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GEORGE MILLER STERNBERG, 1838-1915[1]

By HOWARD A. KELLY

George M. Sternberg, Surgeon General of the United States Army, reorganizer of the U. S. Army Medical Corps and eminent bacteriologist, was born June 8, 1838, at Hartwick Seminary, Otsego County, New York. He was the eldest son of the Reverend Levi Sternberg a descendant of an old Lutheran family of the Palatinate, which had settled in Schoharie and the Mohawk Valley—one Lambert Sternberg (we are told) having been the first to sow wheat in Schoharie County in 1713. His mother, Margaret Levering, whose maiden name was Miller, was an excellent linguist. When he was ten years old George went to live with his grandmother at Hartwick, where two years later his father became principal of the Theological Seminary.

At thirteen he took a situation in a book store, which he held for about a year, spending most of his spare time in reading fiction. At sixteen the *res angusta domi* of the clergyman's family drove George to teaching school at $10 a month and board, some twelve miles from home. Later he secured a school at $20 where he taught for two years, his salary being gradually increased to $100 a quarter. With money saved he returned to his studies at Hartwick, where he at the same time taught mathematics, chemistry and natural philosophy. Having made up his mind to take up medicine he began his preparatory studies under Dr. Hiram Lathrop of Cooperstown, N. Y. He attended his first course of lectures in 1859-60 in Buffalo, N. Y. and later on went to the College of Physicians and Surgeons in New York City, graduating in the spring of 1860. He at once settled in Elizabeth City, N. J., and practised there until the Civil War broke out.

In May, 1861, he entered the Federal Army as Assistant Surgeon and was sent to the Army of the Potomac under General George Sykes. At Bull Run he was captured but escaped later and after sundry vicissitudes made his way to Washington. He was with Sykes until August, 1862, when he suffered from an attack of typhoid fever. After his recovery, for a short time he held the position of executive of the U. S. General Hospital at Portsmouth Grove, R. I., a hospital of 2200 beds.

In November, 1862, he joined General Banks and served as Assistant to the Medical Director of the Department of the Gulf and with the Board of Health until January, 1864. He then became Assistant Medical Director at Columbus and

[1] George Miller Sternberg. A Biography. By his wife, Martha L. Sternberg. Chicago, American Medical Association, 1920.

later was put in charge of a large general hospital in Cleveland, Ohio, whence he was transferred to Jefferson Barracks in July, 1865. During the war he was brevetted Captain and later Major.

In October, 1865, he married Miss Louise Russell of Cooperstown, N. Y.

From Jefferson barracks, in April, 1866, he was ordered to Fort Harker. Here cholera broke out in a colored regiment; it spread to the civil population and Mrs. Sternberg died of the disease July 15, 1867. The sanitary conditions in and around were deplorable, as was noted by Sternberg's report.[2] His habits of close observation are already apparent in this early memorandum: In it he says: "There have been an unusual number of flies and mosquitoes. Houses have been infested with a large fly which differs from the common house fly."

After being on leave from August to December, 1867, he was ordered to Fort Riley as Post Surgeon and on court martial duty. He saw some Indian campaign service under Major M. H. Kidd in 1868.

In September, 1868, he was assigned as chief medical officer to Col. A. Tulley's expedition against hostile Indians about the Arkansas River, with bases near Fort Dodge, Fort Hayes, Kansas, and Camp Supply, I. T.

In December, 1868, he joined Major General Sheridan, in the field depot and headquarters, on the North Canadian River at the junction of Beaver Creek, I. T., dealing with hostile Cheyennes and other tribes. Here he collected crania and fossils, some of which were sent to Joseph Leidy of Philadelphia, and others to the Army Medical Museum.

After being relieved from Sheridan's command March 2, 1869, he was sent for a short while to Fort Hayes, Kansas, but in the same year returned to Fort Riley. Here he had the advantages of a good post with a fine hospital and a thriving town, Junction City, two miles away, where he could obtain photographic materials. Here he married Martha L. Pattison, daughter of Thomas Thurston Nelson Pattison of Scotch-Irish ancestry, a pioneer in Indiana from the Eastern Shore of Maryland, and of Huguenot descent on the side of her mother, Elizabeth Grant Mauzy. At Fort Riley Sternberg indulged his penchant for invention and devised and perfected an anerometer, which, however, the patent office decided had been anticipated by a German. He also invented a practical heat regulator by which an even temperature could be maintained in the wards. Some years later he received $5000 for this device, which in principle is still in use.

In June, 1870, Dr. and Mrs. Sternberg arrived at Governor's Island, where in September an epidemic of yellow fever broke out. During this trying period he rendered faithful service and began his epochal studies of this disease.

After a short service at Fort Hamilton and Fort Warren in July, 1872, he was sent to New Orleans where yellow fever was then prevalent.

In September, 1872, he was assigned for duty to Fort Barrancas, nine miles from Pensacola, where he stayed for three years. Here, as everywhere they went, Dr. and Mrs. Sternberg were deeply interested in the botany of their immediate surroundings, and spent much of their spare time studying and collecting plants.

In the summer of 1873 yellow fever broke out at Barrancas and as the only effectual (and in view of present knowledge the most sensible) remedy then known was to move from the infected area to fresh ground, the troops were at once transferred to a camp near old Fort Pickens across the bay, where they remained until the autumn frost. Again in the summer of 1874 another invasion claimed numerous victims. These epidemics were made the subject of a thorough, intensive study and the conclusion reached that the infection was brought by ship. As a result of these studies Sternberg became our leading authority on yellow fever.

In his spare time he made a study of various shell heaps and mounds in this region and a fine collection of pottery was excavated and sent to a state museum. On this Sternberg made a report before the American Association for the Advancement of Science at Salem, Massachusetts, in 1879.

Another epidemic of yellow fever occurred in June, 1875, brought by the steamship Von Moltke from Havana, in spite of a strict quarantine. At last, Sternberg himself was stricken, but recovered after a severe illness. This was followed by a rest of six months in Europe (1875-76), and promotion to the rank of major, to which he had been entitled since February, 1869.

In May, 1876, he reported at Headquarters, Portland, Oregon, where he utilized the opportunity to become proficient in French.

A side trip to Fort Colville and the Snake River secured some fine fossils, forwarded to Professor Edward D. Cope of Philadelphia.

In 1877 he went through the Lapwai Campaign; he was present at the battle in the Clearwater, and underwent many hardships.

From 1878 to 1885 he was busy experimenting with commercial disinfectants, having only an improvised equipment at his disposal, and pursuing studies in bacteriology, laying sound foundations for the future in these fields in this country.

In 1879 he reported again at Washington, and was detailed for duty with the Havana Yellow Fever Commission of the National Board of Health. He was now able to spend time in intensive microscopic work, with high power lenses, and in making micro-photographs.

In Cuba he became intimate with Carlos Finlay, who was fully convinced that yellow fever was propagated by the stegomyia mosquito. Sternberg's official instructions were to study sanitary conditions, to increase knowledge in pathology, and to investigate endemicity, with some hesitating suggestions as to any elucidation of the fundamental problem of the true nature of the disease. It being generally thought that the organism must be in the blood, Sternberg devoted his time to making micro-photographs and analyzing the records

[2] Surg.-Gen. Office Circular No. 1, pp. 29-30.

of all patients. He made as many as 105 from 41 clear cases, rigging up a heliostat to illuminate a dark room.

In 1880 he returned to Georgetown, D. C. Later in 1880 he went to New Orleans to investigate the micro-organisms of the air in their possible connection with malarial fever. A year before this, Klebs with Tommasi-Crudeli had announced the discovery of a bacillus as the causative factor in malaria; this Sternberg proved to be incorrect.

In 1880 while working on the malaria problem in New Orleans he discovered, by inoculating a rabbit with his own sputum, the micrococcus, which he named *Micrococcus pasteuri*, and which was subsequently demonstrated by Sternberg and others to be the infectious agent in croupous pneumonia. Mrs. Sternberg in her biography is in error in identifying this micro-organism with the Friedländer bacillus. Sternberg was anticipated in publication by Pasteur.

In August, 1881, he was assigned to Fort Mason; here at his own expense he established a laboratory for biological research, and demonstrated the tubercle bacillus discovered by Koch in 1882. His original scientific work was done after the completion of his round of post duties each day.

On November 27, 1883, he wrote Surgeon General Robert Murray summarizing his post work, and begging for better surroundings and facilities for scientific investigation. As a result in April, 1884, he was transferred to the Department of the East, where he was appointed Attending Surgeon and Examiner of Recruits at Baltimore, and found laboratory facilities in Newell Martin's laboratory of The Johns Hopkins University. Culture bouillon and media were made for him by Mrs. Sternberg, who maintained throughout an active and intelligent interest in all his studies.

In 1885 he went as Delegate for the United States to the International Sanitary Conference in Rome, where he was made honorary member of the Royal Academy of Medicine.

Returning in March, 1885, he demonstrated, at The Johns Hopkins University, the living motile plasmodium of malaria discovered in 1880 by Laveran.

In 1886 Dr. with Mrs. Sternberg went to Berlin, where they met Koch; on their return he received the Bausch and Lomb prize for his work on the practical value of disinfectants, begun in Walla Walla in 1878 and completed in the laboratory of The Johns Hopkins University, the culmination of a series of studies undertaken as chairman of a committee of the American Public Health Association.

In May, 1887, he and Mrs. Sternberg went to South America to investigate certain alleged discoveries relating to yellow fever, that of Dr. Freire of Rio de Janeiro, and that of Dr. Carmona de Valle, of Mexico City. The claims of both these workers were shown by Sternberg to have no scientific data to justify them.

On his return from this mission he again located in Baltimore and resumed work with William H. Welch and Wm. T. Councilman in the pathological laboratory of The Johns Hopkins Hospital.

In April, 1888, he asked to be sent to Havana to continue his yellow fever studies. In the course of these he was able to prove conclusively that Gibier's organism was not the cause of yellow fever.

The summer of 1889 saw him once more at work in Havana; this time his wide bacteriologic knowledge enabled him to show that Carlos Finlay's organism, *Micrococcus tetragenus febris flavae* was merely a common non-pathogenic tetrad tropic skin coccus, which often accidentally contaminated cultures. About this time he originated the alkaline treatment of yellow fever.

In 1890 he published his researches on the "Etiology and Prevention of Yellow Fever."

In October, 1890, he was again assigned to San Francisco, where he pursued his scientific studies, worked on his Manual of Bacteriology and lectured on preventive medicine and sanitary conditions.

In February, 1892, he returned to New York as Surgeon and Examiner of Recruits; it was at this time that Mrs. Sternberg isolated a new protozoon in Brooklyn which was named after Dr. Hoagland.

In September, 1892, during the Hamburg cholera epidemic, Dr. Sternberg was called as an authority into active and most successful coöperation in the quarantine service. His "Disinfection at Quarantine Stations, Especially against Cholera,"[3] and other papers written at this time were full of practical interest.

On May 30, 1893, he was made Surgeon General of the United States Army. This high position of responsibility increased his powers for service, and one act taken promptly and with far-reaching consequences was to put Walter Reed in the pathological laboratory of The Johns Hopkins Hospital for advanced study, preparing the way for his great work. At General Sternberg's recommendation the Army Medical School in Washington, D. C., was established June 24, 1893, designed to instruct approved candidates for the Medical Corps of the army in their duties as medical officers. The faculty consisted of: 1. A president of the Faculty, lecturing on duties in war and peace; 2. A professor of Military Surgery; 3. A professor of Military Hygiene (Sanitary); 4. A professor of Clinical and Sanitary Microscopy.

In 1893 he was appointed physician to President Cleveland when in Washington, *vice* his regular physician Dr. Joseph D. Bryant of New York. As Surgeon General he made all possible efforts to advance the interests and standing of the Medical Corps, and yet, with a strange lack of foresight, our House of Representatives in point of fact slaughtered his Medical Corps by reducing its numerical strength from 125 to 110, without making any provision for employing acting Assistant Surgeons. The sanitary atrocities of the troop stations in the Spanish American war were due to the utter lack of preparation for war of "a proud country" [*sic*], and yet all the data needed for perfect sanitary administration were in the hands of General Sternberg. Is it to be wondered at that he was unable to render them effective among the undisciplined troops served often by poor and ill-trained medical

[3] New York Medical Journal, 1893, xvii, 57.

men, one of the best known of whom scorned the value of carbolic acid and all antiseptics and the dangers contamination? And so it happened that of the 6406 deaths among volunteers and regulars, 5438 were from disease, whereas only 968 fell before the human foe. The experiences of the Civil War were repeated in a far more enlightened age.

The Spanish War afforded an added brilliant illustration of the fact that knowledge and good laws are insufficient unless backed by a healthy informed public opinion and cooperation. The ignorance of the doctors appears in the cases of typhoid fever diagnosed as malaria, dengue fever, indigestion, or not reported at all. " In all regiments the death rate from indigestion amounted to 15 per cent of the completed cases! "

Two great lessons stand out from this war: (1) that a trained Medical Corps, suited to an army of 25,000 men in time of peace, is inadequate to take care of 250,000 men at war; (2) that physicians and surgeons taken from private life cannot suddenly undertake to discharge the duties of trained medical officers to an army in the field.

In 1900, it was due to Sternberg that a board of medical officers, including Dr. Rich and Professor Strong, was appointed to go to the Philippines to study tropical diseases, beriberi, the dysenteries, and diseases caused by intestinal parasites and malarial fevers. Here Strong found that the motile *amœba dysenteriæ* was the cause of the prevailing fatal dysenteries, and isolated a bacillus in some cases, similar to Shiga's (1898). Hookworm disease (1899) was shown by Bailey K. Ashford, graduate of the army school, to be the cause of the anæmia and dropsy so prevalent in Porto Rico, instead of malaria, which up to that time had been held responsible.

In 1900 the crowning event of Sternberg's administration was the discovery of the mode of spread of yellow fever by Walter Reed and his party, established near Havana, Cuba, thus proving conclusively Carlos Finlay's hypothesis, and setting on foot the efficient methods for the extermination of the disease, so brilliantly successful in the hands of that practical genius, Major Gorgas (later to be, in his turn, Surgeon General). These two pieces of work constituted the greatest achievement of American medicine, an accomplishment, as a piece of applied scientific work, equal to anything the world has yet seen. Like Moses, who viewed the promised land from Mount Nebo, so Sternberg had worked and sweated over this great subject for the best part of his busy life; it was the one great prize he coveted and he had held it as it were in his hand, when he carefully discussed and rejected Carlos Finlay's theory and inoculation experiments in 1891; but to enter the land of promise was given not to him but to a subordinate working as head of the commission which he appointed.

In 1901 a tour of inspection of four months was made in the Philippine Islands, by order of President McKinley, to determine health conditions for the army.

On December 8, 1900, he addressed the Philosophical Society of Washington, under the auspices of the Academy of Sciences, on malaria.[4]

[4] Smithsonian Report for 1900.

From May, 1893, to June 8, 1902, when he retired, his years were full of intensive work.

In 1897 he became actively interested with Dr. Geo. M. Kober of Washington, in providing sanitary dwellings for people in moderate circumstances, the design being to replace the wretched alley homes. This was accomplished by first providing improved homes for the better class of wage earners, whose vacated houses were then occupied by those lower down. In this way, standard sanitary homes with individuality were secured at reasonable rentals, with a dividend profit of five per cent.

General Sternberg also was interested in passing a law compelling the proper repair or removal of buildings unfit for dwellings. These activities stimulated extensive private enterprises in the erection of two-story sanitary flats.

He was a charter member of the National Association for the Study of Tuberculosis, and as President of the Society for the Prevention of Tuberculosis in the District of Columbia, from 1908 to 1915, he hammered continuously at the disgraceful conditions tolerated in the national capital by indifferent incompetent congressmen. An opportunity of making an intensive practical application of his life-long work and studies in the field, was afforded him in his appointment by President Theodore Roosevelt with a commission of 14 on a " President's Homes Commission." The result was a pioneer report in industrial hygiene.

At the International Congress on Tuberculosis, in Washington 1908, at the dinner to Robert Koch, he was dubbed affectionately by Koch, the " father of American bacteriology."

In part as a result of his tuberculosis work the death rate in Washington from this disease was reduced from 492 per hundred thousand in 1900, among the colored people, to 312 in 1917, and among the white from 188 to 93.

He died peacefully on November 3, 1915, at the age of 77 years, and his body rests beneath a massive block monument in the Arlington Cemetery. Thus passed away the greatest of all our Surgeon Generals up to his time, " America's pioneer bacteriologist " (Welch), a productive scientific investigator of disease, an accomplished sanitary expert, an administrator who greatly improved the organization and opportunities of the medical corps of the United States Army, a man of sterling character, a generous friend, a great humanitarian, whose greatest quality may perhaps be said to be that of unremitting industry and dogged perseverance in great causes, and who reaped rewards proportionately large.

In conclusion, I should like to emphasize the one blessing in life which Dr. Sternberg himself would have accounted the greatest, which he himself would have written first in any list in golden capital letters. He had a remarkable wife, his survivor, in the fullest sympathy with all his aims, deeply interested and participating in every scientific problem which absorbed him, aiding him more especially in his microscopic work, and at the same time sharing in the pursuits which occupied his leisure hours—music, botany and archæology. It is she who has given us a biography, profoundly sympathetic

and appreciative, yet showing a rare restraint. It recalls to one's mind another wifely biography dealing with a different field of endeavor, namely, that of Henrietta O. Barnett, descriptive of her husband's life and work in Whitechapel, London, where too the biographer seeks in vain to obliterate her own great personality behind that of her husband. Would that life held more such sympathetic intimate cooperative fellowships.

HYDROCEPHALUS IN CHONDRODYSTROPHY

By Walter E. Dandy

(From the Department of Surgery, The Johns Hopkins Hospital and University)

Chondrodystrophy or achondroplasia as a clinical and pathological entity needs no brief. The combination of short arms and legs, long trunk and big head at once stamps the condition as unique. The well-known changes, both gross and microscopic, in the epiphyses of the long bones make a pathological picture which is just as distinctive as that of the clinical manifestations. Though the subjects of this disease have figured in the art of the ancient Egyptians who worshipped the gods Bes and Ptah, both of whom have the characteristic chondrodystrophic stature, and though recognized by every generation as a peculiar class of people with mental and physical shortcomings, the separation of chondrodystrophy into a distinct type of deformity really dates from Parrot's description of this malady in 1878.

Prior thereto, Virchow (1856) had accurately described the disproportion in the size of the head and length of the arms, legs and trunk in dead fœtuses, and regarded the disease as congenital myxœdema. Müller (1860) accurately described the principal deformities and considered them manifestations of cretinism. Winkler (1871) felt himself forced to differentiate the condition from true rickets and called it "rachitis micromelica." DePaul (1877-78) insisted that it was not the same as rickets. The gradual tendency to look upon the disease as distinctive reached its climax with Parrot's contribution. He completely separated the disease as a pathological entity but particularly identified the characteristic phenomena in the living, even in adults, and gave the clinical syndrome the name "achondroplasia," by which it has since been known. Additional evidence by Porak (1891) and Marie (1900) firmly established chondrodystrophy as a distinct clinical entity. It is now generally recognized that this condition has no clinical or pathological resemblance to rickets, cretinism, micromelia, osteogenesis imperfecta or other diseases with which it had so long been confused, though quite a few writers, probably from inexperience, still fail to distinguish rickets from achondroplasia.

Historical, histological, pathological and clinical treatises on chondrodystrophy have been so numerous and so thorough that additional repetitions are not necessary. The reader is referred to the admirable papers by Jansen, Kaufmann, and Siegert for the bibliography, the theories of the causation of the disease, the numerous clinical characteristics and its gross and microscopic pathology.

The purpose of this paper is to present evidence bearing upon the cause of the enlarged head in chondrodystrophy. As noted above, the big head is one of the cardinal features of this disease. The explanations have been many, but proof in terms of necropsy material has been almost entirely lacking. Examinations of the skulls of persons afflicted with achondroplasia have been numerous and the descriptions very minute. A frequent observation in this material is a tribasal synostosis, as a result of which the base of the skull is frequently foreshortened, producing brachycephaly with a receding bridge of the nose. Doubtless this observation is true, but it offers no explanation for the increased size of the head. The prevailing teaching is that the size of the head is only relative and is set off by the reduced stature of the individual; but this explanation is far from satisfactory. In many instances this view may be passed over without more than silent skepticism but usually the head is far too large to conform to any relative standard; *there is an absolute enlargement.*

It is difficult to imagine an absolute increase in the size of the cranium except as a result of pressure exerted by the intracranial contents; any increased pressure must be due to some abnormal intracranial condition. The cause could easily be explained by means of post-mortem examinations of the brain but these are singularly lacking. This is only natural, for the achondroplasia is essentially a lesion of the bones and the changes in the skull would hardly have been considered of different causation than those of the long bones.

In the volume of the "Treasury of Human Inheritance" by Rischbieth and Barrington, devoted to dwarfism, achondroplasia is considered at length. A number of photographs of victims of this disease are shown. One showing a group of dwarfs (Fig. 60) is particularly striking, because of the emphasis on the size of the head in the legend beneath, which reads as follows: "Large group of ateliotic cases and four cases of achondroplasia. *The latter may be recognized by their large heads and adult faces.*" But no explanation of the enlarged head is offered. The head of an ateliotic dwarf is in proportion to the rest of the body; the head of an achondroplastic dwarf is disproportionately large.

Virchow first called attention to the shortening of the base of the skull although, of course, he did not conceive of achondroplasia as a distinct disease, regarding it as akin to fœtal rickets or cretinism. Though he explained the shortening by a premature union of the tribasal bones, whence the name "tribasal synostosis," the real cause was found by Kaufmann and substantiated by Jansen and others to be a lack of development of the bones at the base of the skull, as of the other bones

of the body. In a series of 13 cases, Kaufmann found, without exception, this shortening of the base of the skull. Parhon, Shunda and Zalplachta called attention to the increased breadth of the skull in a number of cases taken from the literature. Taking the normal breadth of the skull to be 80 per cent of the length, they found the brachycephalic index to be 81, 83, 87, 88, 94, and 100 in the series. They also noted an increased circumference of the head and made the important observation that *the intellect is inversely proportional to the circumference of the head.* Porak and Durante, also, noted that the head is often enlarged in chondrodystrophy. Jansen makes mention of hydrocephalus in achondroplasia, saying, "the fact of the abnormally great circumference in other achondroplasts and in two cases of hydrocephalus among our five cases can be no mere accident." This is the most impressive statement and, supported by the best evidence of the existence of hydrocephalus that has come to my attention. In explanation of the hydrocephalus, he made the advanced hypothesis that the aqueduct of Sylvius or another part of the ventricular system was probably compressed and consequently the outflow of fluid was impeded. He mentioned, also, the possibility of vascular disturbances causing an increase of fluid by distortion of the vessels.

In an exhaustive treatise on hydrocephalus by D'Astros, there is a most interesting chapter on "Rachitisme céphalique," in which he concludes that an indisputable relationship exists between rickets and hydrocephalus. Bourneville supported the views of D'Astros and considered at least half of all hydrocephalic children to have rickety deformities of the thorax and vertebral column and assumed that rickets played some part in the production of the hydrocephalus. D'Astros admits the view that, in the great majority of cases of rickets, the cranial chamber is not larger than normal but he still thinks there is an increase in the amount of cerebrospinal fluid. He mentions the description of hydrocephalus in rickets by Glisson in 1651. Unfortunately, these authors had not differentiated rickets from achondroplasia. D'Astros shows a photograph of a typical case of chondrodystrophy with hydrocephalus; this photograph, which is given for a case of rickets, is reproduced here (Fig. 6). Undoubtedly, the incidence of hydrocephalus in chondrodystrophy would be much greater were the cases of ordinary rickets excluded, and it is evident why the arguments of these authors were unavailing in the absence of absolute proof from examinations of the brain. Many authors (Cestan, Swaboda, Kassowitz, Baylord, Lecourtois, Regnault, Marfan, Durante) claim that the large head is not hydrocephalic, that it is the bones that are hypertrophied, and that the size of the intracranial chamber is not increased. It should be mentioned here that there has never been any proof that an actual increase in the thickness of the bones of the skull exists.

The only record of a necropsy of the brain which I have found in the literature was by MacCallum. He remarked in the course of a thorough necropsy report "On section, the cerebral ventricles were found markedly dilated with clear fluid." No cause of the hydrocephalus was found and no explanation offered. The patient was 75 years old.

Recently, a boy of 19, with all the typical features of chondrodystrophy, entered the surgical service of The Johns Hopkins Hospital for the correction of bow-legs. The very large head was the most striking feature of the patient. It was out of all proportion to the rest of his dwarfed body and even when his body was concealed, the size of the head was scarcely less impressive. Moreover, his intelligence was far below normal.

My interest in chondrodystrophy was stimulated by this seeming paradox—a cranium larger than normal, but a defective mentality—paradoxical only in the light of the usual explanations offered. Were the brain of normal size or by some impossible chance hypertrophied so as to fill the increased cranial chamber, why should the function of the mind be reduced? There must be some simple relationship between the size of the head and the mental deficiency. There is only one condition which could, at the same time, cause the head to enlarge and reduce the mental capacity and that is one of the most common of intracranial affections—hydrocephalus.

The patient's mentality is little greater than that of a boy of six or seven. His large head, short arms and legs, and relatively long trunk are shown in the accompanying photographs. He is the third child; the other two being normal. Although the labor was very difficult, and the head unusually large, instruments were not used. The mother, who is very intelligent and observing, volunteered the information that for a long time after birth the head was soft and had not the resistance of a normal baby's head; it was heavy and not until the patient was two years old could the head be held up. The anterior fontanelle was open until the fourth or fifth year. Eight years ago, (the patient was then 11 years old), he wore a 7½ size hat. The head grew somewhat after that, but for several years there has been no growth. It is impossible to get hats to fit him; his mother makes his caps of stocking material. He learned to walk between the third and fourth year. He never crawled, but learned to move himself by rolling. He was three years old before he could talk but his speech was perfectly distinct and not abnormal. During the first three years, the patient was delicate, very restless and irritable and whined a great deal. Noises disturbed him and music made him unhappy. Teething began at the sixth month and continued at the normal rate.

His memory is good in some ways. He carries out errands perfectly well. He can tell the ages of all the members of the family and remembers telephone numbers very well, even those that are used only occasionally. He was a good speller and a good reader in school, but was poor in penmanship, geography, history, and mathematics. He can add, subtract, multiply, and divide very well but cannot grasp anything more complicated. He was in the sixth grade at school when he stopped, at the age of 18, but his mother thinks he had been pushed along a little faster than he should have been and was hardly capable of carrying work so advanced He plays with boys but gets along better with the older ones. He is quite sensitive about his stature and does not like to be teased. On the whole,

his disposition is very good. He is usually kind and agreeable, only occasionally showing evidences of temper.

Before the child was a year old, the family doctor told the mother that he had water on the brain. It was not noticed that his arms and legs were shorter than they should have been until he was 14 or 15 years of age. His growth has always been stunted but the mother thought "the condition of his head kept him small." Until three or four years of age, the patient was troubled with enuresis, only at night, but the mother says that one of her other children has had this trouble just as badly. He is subject to transitory attacks of unconsciousness, though without convulsions. At present, he delivers packages and is quite reliable.

The examination reveals a dwarf, 130 cm. in height, with the proportions as evident in the photograph. The circumference of the head is 65 cm. as contrasted with the normal of 54 cm. for his age. His epiphyses show delayed development, having the appearance in the roentgenogram of those of a boy of 14 (Dr. Baetjer). There is a bilateral genu valgum. The hand is the characteristic *main en trident*. The elbows cannot be extended beyond an angle of 160°. The Wassermann reaction of the blood is negative, nor is there any history of syphilis in the family. The genitalia are normal. No history indicating sexual precocity was elicited.

Evidence of Hydrocephalus by Ventriculography

The determination of hydrocephalus and of its degree can be determined just as accurately by ventriculography as by a post-mortem examination of the brain. Through a ventricular puncture in the right occipital region, 350 c. c. of fluid were removed from the right lateral ventricle and an equal quantity of air was injected. Doubtless, 100 to 200 c. c. of fluid, or even more, could easily have been obtained, had I attempted to empty the ventricles completely, but, as it was only desirable to get a picture of one ventricle in profile and a view of both ventricles in cross-section, the removal of all the ventricular fluid was unnecessary.

It will be seen from the ventriculogram that the greater part of the intracranial chamber is filled with air. The picture of the enlarged ventricles is pathognomonic of hydrocephalus. But the ventriculogram shows a great deal more. It shows that the hydrocephalus is no longer progressive; it has been arrested. This conclusion is reached because the air, which has been injected into the ventricle, has reached the farthermost radicles of the subarachnoid space—the cerebral sulci, which are shown as numerous wavy lines criss-crossing the roentgenogram. In other words, the air has had a free passageway from the lateral ventricle, through the third ventricle, the aqueduct of Sylvius, the fourth ventricle, and foramina of Luschka and Magendie into the cisterna magna and the other cisternæ under the brain, and it has finally passed along the numerous primary branches and reached the cerebral sulci. It is here that cerebrospinal fluid is absorbed. If fluid (or air) reaches *all* or even many of the cerebral sulci from the ventricular system, it is proof that hydrocephalus cannot develop and, if the ventricles are already large, that the disease cannot be progressive. In those cases of hydrocephalus in which we have relieved an intraventricular obstruction, whether from a tumor or an inflammatory process, the patency of the newly constructed opening can be demonstrated by the passage of air through it.

Further evidence that the subarachnoid space is yielding adequate absorption of cerebrospinal fluid in this case was shown by the quantitative absorption of phenolsulphonephthalein after its injection into the spinal canal. An absorption of 30 per cent was obtained during the first two hours following the injection. This is rather low but within the limits of normal. Furthermore, the colored fluid introduced into the spinal canal was later freely obtained from a lateral ventricle, thereby demonstrating the patency of the foramina of Luschka and Magendie, the aqueduct of Sylvius and the foramen of Monro. It should be noted that no convolutional atrophy of the skull or separation of the sutures is evident in the roentgenogram.

Cause and Spontaneous Cure of the Hydrocephalus

From the accurate history given by the mother—the large head causing difficult labor at birth (the mother was a multipara and the child presented by the head), the large fontanelle with delayed closure (3rd to 4th year), the delay in holding up the head and in beginning to walk—there is unmistakable evidence that the hydrocephalus was present at birth and was progressive during the early years of life. Apparently, it increased until the 12th or 13th year, the time at which the mother noticed that his head ceased to grow. During all these years, however, the accumulation of fluid must have been very slow, for had it been otherwise, the head would have assumed tremendous proportions.

In achondroplasia, we are confronted with an unusual type of hydrocephalus. Ordinarily, hydrocephalus is a progressive disease which is only occasionally cured spontaneously. But, apparently, in all the cases of achondroplasia in which the patients live past childhood—and many of them do—the hydrocephalus is arrested but a big head and a defective brain are usually the result. This statement is not intended to imply that necessarily every case with a large head has a defective mentality, for a considerable destruction of the cerebral tissue may and does take place with little or no apparent mental changes. In fact, occasionally we see examples of a brilliant mind and, at times, even evidences of a kind of mental precocity in undoubted instances of hydrocephalus in achondroplasia, or in the other rarer instances of hydrocephalus in which a cure has resulted; but such instances are certainly rare. How, then, can we explain such an unusual type of hydrocephalus, occurring so constantly in achondroplasia and tending toward a spontaneous cure? From a survey of the cases in the literature, I believe there is a definite relationship between the size of the head and the intensity of the other bony changes in achondroplasia. It is generally conceded that in the most severe grades, and they comprise the greater number, the

children die *in utero* or at birth. In these severe grades, the extremities may be small nubbins on a long trunk and the head will usually be large, though even a high grade of hydrocephalus at birth is not always accompanied even by a striking enlargement of the head.

Two inferences are possible—if such a relationship can be proved to exist, either the hydrocephalus causes the changes in the bones, or the changes in the bones cause the hydrocephalus. It is not conceivable that injury of the brain could be responsible for the bony changes, for in hydrocephalus of other types, of all grades, and beginning either in the prenatal period or after birth, there are no such tendencies toward dwarfism or to the production of micromelia. In some way, it would therefore seem that the hydrocephalus is probably secondary to the bony changes of achondroplasia. The primary changes in the bones of the skull have been recognized since the findings of Virchow, though, as mentioned above, the explanation of the findings has been materially altered. A distinct shortening of the base of the skull is probably present; it is doubtless due to osteogenetic deficiency, and is exactly comparable in its origin to the bony changes which result in the shortening of the humerus, femur and other long bones (the membranous bones apparently being exempt). That this relationship exists, I do not doubt, but its explanation lacks the evidence of clinical studies of cases and necropsies. It is conceivable that such a shortening of the base of the skull may compromise the growing brain as to volume, but more vitally as to position. A certain degree of kinking or bending of the brain-stem should follow, and it is possible or even probable that thereby the lumen of the aqueduct would be reduced, producing a partial occlusion. It is also possible that the shortening of the base of the skull may similarly obstruct the cisternæ under the pons and midbrain, and as the cisternæ form the trunk of the tree of the subarachnoid spaces, an occlusion would produce results exactly comparable to those resulting from occlusion of the aqueduct of Sylvius. If either the aqueduct of Sylvius or the cisternæ are affected in this manner, a partial obstruction might result. In the most advanced grades, the obstruction might well be complete. Such an occlusion, either partial or complete, would inevitably cause hydrocephalus by preventing the cerebrospinal fluid from reaching, in adequate quantity, the cerebral sulci, where all the absorption of cerebrospinal fluid is effected. As the size of the skull, particularly of the base, increases, it is conceivable that these occlusions may gradually be automatically corrected. The intracranial pressure resulting from the increased amount of fluid could play such a rôle in correcting the hydrocephalus by stretching the membranous bones of the vault and possibly even those of the base of the skull.

Such an explanation is entirely hypothetical. It is not conceivable that any of the usual obstructions at the aqueduct of Sylvius, either tumors or cicatricial stenoses, could explain the hydrocephalus, because these pathological changes are almost universally progressive and corrections could scarcely result spontaneously. Nor, for the same reason, could the usual inflammatory changes in the cisternæ or at the foramina of Luschka and Magendie produce this type of hydrocephalus.

A second patient (Fig. 4) is also a typical achondroplastic dwarf with an abnormally large head which, undoubtedly, is due to hydrocephalus. It will be noted, however, that the head is not brachycephalic; it might be even classified as dolichocephalic, though the lateral enlargement is almost as conspicuous as the antero-posterior. As a matter of fact, there is no apparent shortening of the antero-posterior diameter of the head in the first case.

The history of the second patient is of little value because of the mother's lack of observation. She does not know, even approximately, when the anterior fontanelle closed, when the patient learned to hold up his head, to walk or to talk. She has two children, but had not observed anything wrong with this boy, although the neighbors frequently called attention to his large head. He seemed perfectly well and strong after birth, which followed a dry labor. There is an added complication, the importance of which it is difficult to estimate. When three years old, the patient had a severe attack of meningitis, with characteristic opisthotonos, cervical rigidity, vomiting, etc. It is well recognized how frequently hydrocephalus follows meningitis, but the mother is confident that the baby's head was large before this illness, and that there was a quick and apparently complete recovery from meningitis. His mother brought him to the hospital because of stunted growth, he not having grown perceptibly in the past two years. He is 102 cm. high, contrasted with the normal of 117.4 cm. for his age. His head measures 62 cm., the normal for seven years being 51 cm. During the past year, he has been under frequent observation at the hospital and during this time his head has grown 3.5 cm. He is now a bright boy; he stands well in his classes at school, ranking tenth in a class of 45 children. He complains of no headaches; but his mother and the teacher at school have had great difficulty in getting him to hold up his head. Whenever possible, he lies down, or when sitting, finds some object upon which to rest his head. The roentgenogram shows a definite increase in the intracranial pressure, evidenced by a mild grade of convolutional atrophy of the skull and by a slight separation of the cranial sutures. A cracked-pot sound is clearly demonstrable. In the preceding case, this atrophy of the skull is absent because the growth of the hydrocephalus has been arrested for some time. In this case, we have no absolute proof of the existence of hydrocephalus, which is demonstrated in the other case, though there can be no reasonable doubt, from the facts just enumerated, that it must be present.

Treatment of the Hydrocephalus

Naturally, the most serious aspect of chondrodystrophy is the mental impairment caused by the hydrocephalus. Can this be remedied in any way? Since the introduction of the newer methods by which the diagnosis and localization of intracranial lesions are made possible, cures of hydrocephalus are now frequently obtained by correction of the cause. I am confident that a careful study of each case of hydrocephalus in

FIG. 1.—This boy of 19 years is the victim of achondroplasia. His head is large, not only relatively but absolutely. This increased volume of the head is due to hydrocephalus. The history of the patient is given in the text. The ventriculogram showing the degree of hydrocephalus is shown in Fig. 9. The intelligence of this boy is greatly reduced.

FIG. 2.—Enlarged profile view of head of the same achondroplastic boy to show shape of head.

FIG. 3.—Enlarged front view of same patient.

FIG. 4.—Another boy of eight years with typical achondroplasia. His head is relatively larger than that of the preceding patient. His intelligence is still normal but the head is increasing in size. A ventriculogram was not obtained, but the existence of hydrocephalus can scarcely be doubted, especially after seeing the ventriculogram of the preceding case.

FIG. 5.—Profile view of same child.

FIG. X. —Rachitisme céphalique. —Ar . . ., 12 ans (obs. VI). Cet enfant a la taille d'un enfant de sept ans. Cette petite taille qui contraste avec le volume exagéré de la tête est due surtout au rachitisme des membres inférieurs.

FIG. 6.—This photograph and legend are copied from D'Astros' book in which he considers a form of cephalic rickets. The picture is that of a typical achondroplasiac with the characteristic large head, short arms and legs and long trunk.

FIG. 7.—Ventriculogram of a normal lateral ventricle, lateral view.

FIG. 8.—Antero-posterior ventriculogram of normal ventricles.

FIG. 9.—Ventriculogram (profile view) of the lateral ventricle of the patient shown in Fig. 1. The air has not filled the entire ventricle but the tremendous enlargement of the ventricle is strikingly demonstrated. The thickness of the cerebral cortex is of course correspondingly reduced.

FIG. 10.—Antero-posterior ventriculogram of the same case of [illegible] droplasia (Fig. 9).

achondroplasia by these methods will accurately define and locate the cause of hydrocephalus and thereby indicate a rational form of operative treatment which may stop the disease and prevent the cerebral destruction with its resulting mental impairment. The second case is an example in point. Undoubtedly, the hydrocephalus is still progressing; a growth of the head of 3.5 cm. in a year is sufficient indication. The separation of the sutures and the convolutional atrophy of the skull make this almost certain. At present, his mind is not noticeably impaired, but, in time, mental impairment is inevitable unless the progress of the hydrocephalus soon ceases. In order to locate the site of the cause of his hydrocephalus, he should be tested by intraventricular and intraspinous injections of air, and by the phenolsulphonephthalein intraspinous test, to determine the quantitative absorption from the subarachnoid space and also the presence or absence of organic obstructions in the ventricular system. Since it is now possible to detect with such accuracy where an obstructive lesion lies, to tell whether an occlusion is partial or complete, and to determine whether and to what degree the subarachnoid space is absorbing cerebrospinal fluid, we should not permit the destructive sequelæ of hydrocephalus to develop until every possibility of cure has been eliminated. After the cause of the hydrocephalus has been determined, it is hoped the cure of the disease may be possible and in the earlier stages, instead of awaiting the usual spontaneous correction at which time the mentality is usually irreparably impaired.

CONCLUSIONS

1. The large head in our cases of chondrodystrophy has been shown by ventriculography to be due to hydrocephalus. The large heads of other recorded cases presumably have a similar cause.

2. Hydrocephalus in achondroplasia differs from other types of hydrocephalus in that its development tends to cease spontaneously. In some instances, at least in the two cases here reported, it progressed very slowly and for a long period before arrest eventually took place.

3. When untreated, a defective brain, it would seem, inevitably results.

4. We have had no opportunity to observe the progression of hydrocephalus, but it may be possible, by the newer methods of intracranial study, to ascertain the cause and possibly avert the disastrous sequelæ.

5. The size of the head and, therefore, the grade of hydrocephalus seems to be proportionate to the severity of the dwarf phenomena in chondrodystrophy.

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THE MECHANISM OF THE CARRIER STATE, WITH SPECIAL REFERENCE TO CARRIERS OF FRIEDLÄNDER'S BACILLUS

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During the course of experiments on the fate of bacteria introduced into the upper air passages, we became interested in the question of the mouth as an environment for bacterial growth. The rapid disappearance of foreign organisms after experimental introduction suggested that the free surfaces of the buccal mucous membranes, when intact, were unfavorable for the colonization and growth of extraneous bacteria.[1] As a corollary of this conclusion the question arose whether in the so-called carrier state the organisms are confined solely to some acute or chronic focus of diseased tissue whence they are discharged to the free surfaces of the mouth and throat, or whether the organisms actually live on and grow diffusely over these surfaces.

We have found no experimental evidence bearing on this point, but clinical observations suggest the great importance of a focus of infection in the mechanism of the carrier state. A brief review of these facts may be presented before detailing our experimental observations.

Clinical Observations on the Localization of Bacteria in Chronic Carriers

In the present discussion we shall consider only so-called chronic carriers. By this term we mean individuals who harbor, more or less constantly, in the upper air passages, some pathogenic organism, either following disease, or without any history of previous disease due to the organism in question. A few of the main types of such carriers may be mentioned to illustrate the principles involved.

1. *Diphtheria Carriers.*—The persistence of positive cultures in many instances following diphtheria is a matter of common experience. Often the organisms are present for indefinitely long periods—months or even years. In such cases they not uncommonly disappear following tonsillectomy, when all other measures had previously been unavailing. The fact that cultures made from the depths of the crypts or from beneath the capsule of the excised tonsils show numerous diphtheria bacilli suggests that these glands were the breeding place of the organisms, and that they were not growing and multiplying on the free surfaces of the normal mucous membranes.

2. *Streptococcus Carriers.*—The presence of virulent hemolytic streptococci in the throats of healthy people has been frequently noted, especially at times when streptococcus infections are prevalent. Although the organisms may occasionally be obtained from the throat or nose, it is found in general that a much higher percentage of positive yields results if cultures are made from tonsil crypts. Thus, Pilot and Davis[2] found β hemolytic streptococci in almost 100 per cent of excised tonsils. Furthermore, it appears that in streptococcus carriers tonsillectomy is often followed by the disappearance of the organisms from the throat (Tongs,[3] Van Dyke[4]). These observations suggest that here again the tonsil often is the breeding place of the streptococcus whence it is discharged to the free surface of the throat without actually colonizing there.

3. *Meningococcus Carriers.*—In a variable number of normal individuals meningococci are found on repeated cultures. Studies such as those of Herrold[5] show that a much larger percentage of positive cultures can be obtained from the nasopharynx than from the tonsils, nares, or sputum. Furthermore, the application of disinfectants directly to the nasopharynx seems to lead to the clearing up of carriers more promptly than treatment of other parts of the upper air-passages.

In summary, then, the weight of clinical evidence in the case of carriers of important pathogens tends to show that the organisms actually colonize in a focus of diseased tissue in the upper air-passages whence they are discharged to the free surfaces. It seems probable that bacteria do not actually localize and grow upon the latter, but that they are constantly washed away only to be replaced by other organisms freshly discharged from the focus. We have been unable, however, to find any actual experimental proof or disproof of this idea.

In the course of some studies of Friedländer bacillus carriers, methods by which this point might be demonstrated suggested themselves, and the present paper deals with these observations. They are based mainly on an intensive study of three carriers whose histories are appended in some detail.

Case I.—G., aged 50, colored, male.

Diagnosis.—Syphilis, aneurism of aortic arch, aortic insufficiency, myocardial insufficiency.

History.—No history of severe colds, of sinus infection, tonsillitis, cough or pneumonia.

Examination.—Nose, negative except for racial hypertrophy of turbinates. Tonsils, moderately enlarged and injected. Pharynx, negative. Larynx, negative. Ears, negative.

CASE II.—W. J., aged 31, male, colored.

Diagnosis.—Acute nephritis.

History.—Frequent colds but no sore throats or tonsillitis. No pneumonia. No cold "this year."

Examination.—Nose, anterior ends of middle turbinates look normal. There is no discharge. Tonsils, embedded. There are numerous plugged crypts on each side. Pharynx, negative. Naso-pharynx, a moderate amount of adenoid tissue is seen. Ears, drums intact, no retraction. Sinuses, clear on transillumination.

CASE III.—J. H. Hosp. Med. No. 42,846, white, aged 34.

Diagnosis.—Chronic rheumatic endocarditis, aortic and mitral insufficiency.

History.—No history of severe colds, sore throats, sinus infections, or pneumonia.

Examination.—Nose, septum deflected to right causing partial nasal obstruction. Small amount of discharge on both sides. Pharynx, clear. Ears, marked grade of retraction of drums on both sides. Tonsils, moderately enlarged and adherent. Naso-pharynx, polypoid inferior turbinate posteriorly on right. Eustachian orifices clear. No discharge from posterior sinus orifices.

Incidence of Friedländer Bacillus Carriers.—Eighty-five unselected individuals were examined. The Friedländer bacillus was isolated from the throats of five—a percentage of 5.8.

Persistence of Friedländer Bacilli in Carriers.—In every instance the carrier state persisted during the entire period of observation (Table I). We have no idea of its previous or subsequent history.

TABLE I.—PERSISTENCE OF FRIEDLÄNDER'S BACILLUS IN CARRIERS

Name	Period of observation	Number of cultures	Number positive	Number negative	Last culture
G.	42 days.	16	16	0	Positive.
J.	36 days.	12	12	0	Positive.
W.	Four months.	15	13	2	Positive.
H.	Three months.	16	14	2	Positive.

Spread of Friedländer Bacilli from Carrier to Contacts.—It seemed of interest to determine whether there was any tendency for contacts to acquire the organisms from the carriers and possibly to develop a carrier state themselves. Patient W. was in the ward for several months. During this period 285 throat cultures were made on 31 other patients in more or less close contact with him. In no instance was Friedländer's bacillus recovered. Subsequently more careful contact observations were made on two other patients. The carrier and the contact were placed in adjacent beds. No attempt at isolation was made and they used the same bed-table and to some extent the same utensils. Cultures were made daily from the throats of contact and carrier (See Table II). At no time were Friedländer bacilli recovered from the contacts in spite of their persistance in the carrier. These observations agree with our experiments on the rapid disappearance of these organisms when experimentally introduced into the mouth in large numbers and afford an interesting contrast to the rapid spread of an organism which is producing disease

TABLE II.—SUMMARY OF CULTURES FROM FRIEDLÄNDER BACILLUS CARRIERS AND FROM CONTACTS

	Bed 1 Carrier G Feb. 17.	Bed 2 Carrier J March 20	Bed 3 Contact T March 20
Culture on March 23.	+	+	0
" 24.	+	+	0
" 25.	+	+	0
" 27.	+	+	0
" 29.	+	+	0
" 30.	+	+	0
		Contact T March 30	
" 31.	+	0	
April 1.	+	0	
" 3.	+	0	
" 5.	+	0	
		Contact W April 6	
" 7.	+	0	
" 8.	+	0	
" 9.	+	0	
" 12.	+	0	
	Carrier J April 1	Contact C April 5	
" 6.	+	0	
" 7.	+	0	
" 8.	+	0	
" 9.	+	0	
" 10.	+	0	
" 11.	+	0	
" 12.	+	0	

such as occurs, for example, in epidemics of streptococcus infection.

Site of Localization of the Friedländer Bacilli in Carriers.—Cultures were made from various parts of the upper air-passages to determine the relative number of organisms present. As appears in Table III, while the organisms were found constantly in the pharynx in large numbers, a few were occasion-

TABLE III.—RESULTS OF CULTURES MADE FROM VARIOUS PARTS OF THE UPPER AIR PASSAGES IN FRIEDLÄNDER BACILLUS CARRIERS

Case	Date of cult.	Nares, right	Nares, left	Throat	Right tonsil	Left tonsil	Pharynx
G.	Mch. 27.	No F.	No F.	13 cols. F.	—	—	—
	" 29.	No F.	No F.	∞ F.	—	—	—
	Apr. 4.	2 cols. F.	No F.	∞ F.	—	—	—
	" 5.*	No F.	1 col. F.	∞ F.	—	—	—
	" 6.*	No F.	No F.	—	100 cols. F	No F.	No F.
	" 8.*	No F.	No F.	—	∞ F.	No F.	No F.
J.	Mch. 27.	No F.	∞ F.	∞ F.	—	—	—
	" 29.	No F.	No F.	∞ F.	—	—	—
	" 31.	No F.	No F.	∞ F.	—	—	—
	Apr. 8.*	No F.	No F.	—	3 cols. F.	13 cols. F.	4 cols. F.
	" 9.*	No F.	No F.	—	∞ F.	6 cols. F.	22 cols. F.
	" 11.*	5 cols. F.	No F.	—	∞ F.	No F.	4 cols. F.

* Throat thoroughly gargled with water before cultures were made.

ally recovered from the nares as well. The sequence of cultures seems to show that such organisms are introduced accidentally from the pharynx, and are promptly washed away without localizing on the nasal mucosa. Further differential cultures in two cases also showed that the organisms were constantly present on one tonsil. The tonsil, therefore, seemed to be the focus from which the bacteria were disseminated through the buccal cavity.

Re-Implantation of Carrier's Strain on His Own Mucous Membranes.—With the above observations in mind, it seemed of interest to re-introduce the carrier's own strain upon his own mucous membrane. It seemed that this might give information as to whether these surfaces behave like those of normal individuals in whom the organisms introduced are promptly washed away, or whether, in the carrier, the organisms have adapted themselves to a free growth on the open surfaces of the upper air-passages. It appeared (Table IV)

TABLE IV.—INTRODUCTION OF CARRIER'S OWN STRAIN UPON HIS OWN MUCOUS MEMBRANES

Name	Date	Culture, right nose	Culture, left nose
G.	April 4.	2 cols. F.	No F.
	One loop of solid growth B. Friedländer—Strain G—inoculated on left nasal septum.		
	Culture immediately.	No F.	F.
	Culture after 1 hour.	No F.	F.
	Culture after 24 hours.	No F.	No F.
	Culture after 48 hours.	No F.	No F.
J.	March 29.	No F.	No F.
	One loop of solid growth B. Friedländer—Strain J I—inoculated on right nasal septum.		
	Culture immediately.	F.	No F.
	Culture after 1 hour.	F.	No F.
	Culture after 1 day.	Several hundred cols. F.	No F.
	Culture after 2 days.	No. F.	No F.

that organisms introduced in this way did not persist, but disappeared at the same rate of speed as the Friedländer bacilli placed on the nasal septum of the non-carrier.[6]

Introduction of a Second Strain of Friedländer Bacilli upon the Mucous Membrane of the Carrier.—It seemed of interest to determine how the carrier would react to the introduction of a second strain of B. Friedländer. Such an experiment was made possible by working with strains sufficiently different to enable one to differentiate the carrier's strain and the organism introduced. Carrier G. harbored an organism which had the following characteristics: Colonies up to 1 cm. in diameter, confluent. Greyish white, slightly opaque, sticky growth—not very stringy. Microscopically: short gram-negative bacilli with moderate capsule formation. Fermentation of sugars (24 hours) saccharose +, dextrose +, mannite +, lactose 0. Carrier J's strain grew with a profuse opalescent very sticky and stringy growth. Short gram-negative bacilli with very marked capsule formation. Fermentation of sugars (24 hours)—dextrose +, mannite +, saccharose 0, lactose 0. When a mixture of these two strains was grown on the same plate it was readily possible to pick out the two different types of colonies. After control cultures had been made, each of these two carriers was inoculated with the other one's strain, and cultures were made at various intervals. The result of this experiment (Table V) is that the foreign strain replaces

TABLE V.—INTRODUCTION OF A SECOND STRAIN OF B. FRIEDLÄNDER UPON THE MUCOUS MEMBRANE OF THE CARRIER

Name

G. March 28—
Control culture before inoculation (pharynx) = ∞ cols. strain G.
A large loopful of strain J. (fished from the original plate) was swabbed on G.'s tongue and pharynx.

Culture made immediately	(pharynx)	= ∞ cols. strain J. — 6 cols. G.
" " after 2 hrs.	"	= ∞ cols. strain G. — no cols. J.
" " " 24 hrs.	"	= ∞ cols. strain G. — no cols. J.
" " " 48 hrs.	"	= ∞ cols. strain G. — no cols. J.

J. March 28—
Control culture before inoculation (pharynx) = ∞ cols. strain J.
A large loopful of strain G. (fished from original plates) was swabbed on J.'s tongue and pharynx.

Culture made immediately	(pharynx)	= ∞ cols. strain G. — no cols. J.
" " after 2 hrs.	"	= many cols. strain G. — many cols. J.
" " " 24 hrs.	"	= many cols. strain J. — no cols. G.
" " " 48 hrs.	"	= ∞ cols. strain J. no cols. G.

the carrier's strain in the cultures for a few hours, but promptly disappears, so that after 24 hours only the carrier's strain is recovered. In other words a carrier reacts to the introduction of a second strain of B. Friedländer just as a non-carrier does. This experiment seems to show then that there is no special alteration in the mucous membranes of the carrier which makes them a suitable medium for the growth of Friedländer bacilli in general.

Attempts at Carrier Production.—An attempt was made artificially to produce a carrier state by frequent re-inoculations. An individual (B), was inoculated by swabbing the tongue with a freshly isolated strain of B. Friedländer. Cultures were taken at various intervals following inoculation and daily reinoculations made with these cultures. This process was repeated daily for one week. Within 24 hours after the last inoculation this man was free from B. Friedländer. This result was just what had been expected and lends some support to the view that a focus of infection is responsible for the carrier state in the case of this organism.

Discussion

The present report, in summary, presents experimental evidence on the mechanism of the carrier state, at least in certain instances. It has been possible to show in the case of these Friedländer bacillus carriers that the breeding place of the bacteria is in a definite focus—the tonsil. From this point the organisms are discharged into the open pharyngeal cavity, and at times may be introduced into the nose. There is no evidence, however, to indicate that any adaptation takes place between the bacilli and the mucous surfaces, leading to actual growth and multiplication on these surfaces. They react just as the normal mucous membranes do, both upon the introduction of the carrier's own strain, or the introduction of a second strain of Friedländer's bacillus.

CONCLUSIONS

1. Of 85 unselected individuals 5.8 per cent were found to be carriers of Friedländer's bacillus

2. The carrier state persisted throughout the period of observation.

3. There was no tendency for contacts to acquire the carrier state.

4. Differential cultures showed the breeding place of the Friedländer bacilli to be in the tonsil.

5. The carrier's own strain or a foreign strain of Friedländer's bacillus implanted upon the free surfaces of the mucous membranes disappeared at the same rate of speed as in a non-carrier.

6. It was impossible artificially to produce a carrier state by repeated inoculation with *B. Friedländer.*

7. The general conclusion from these observations is that the carrier state depends on a focus of diseased tissue which affords a breeding place for the bacteria. They do not become adapted to growth on the free surfaces of the mucous membranes.

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TUBERCULOSIS OF THE KIDNEY IN WOMEN

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This paper will deal precisely with the final results of surgical treatment. I shall speak briefly of diagnosis but shall not go into the pathology of the urinary tract, in view of the fact that this has been so well covered in many text-books.

Although the number of our cases is only 77, the record of our results is of interest for the following reasons: (1) The diagnosis in these 77 cases is sure because the specimens obtained from the patients operated upon presented the typical gross and microscopic pictures of tuberculosis of the kidney, and in the cases in which no operative interference was deemed available the urinary findings and cystoscopic findings left no doubt as to the pathological condition from which the patient was suffering. (2) A large proportion of our cases were treated many years ago. One patient, for instance, is alive and well 20 years after her operation, another 22 years, and quite a considerable number after fifteen years. Finally, the method of operating in this clinic has not been uniform, and it is of interest to compare the results obtained by the different methods. In some cases only the kidney was excised while in others a second incision, usually a McBurney, was made and the ureter was removed extraperitoneally down to the uterine vessels; or the excision was complete and included a section of the bladder wall. This simultaneous extirpation of the kidney and ureter was first described in February 1896, by Dr. Howard A. Kelly (The Johns Hopkins Hospital Bulletin, 1896, VII).

A careful study of the histories of our cases has given us the following information: In our 77 cases there was a definite family history of tuberculosis in 14, or 18 per cent. By definite family history I mean that some member of the immediate family had had tuberculosis and do not refer to tuberculosis in distant relatives. In 46 cases the right kidney was affected, in 27 the left , and in 4 the disease was bilateral.

The average age of our patients on admission was 32 years, and the majority had begun to have symptoms in the third or fourth decades. The following table groups the patients according to their ages on admission and in each group the average duration of symptoms before entrance to the hospital is given.

Age	Number of cases admitted to the hospital	Average duration of symptoms before admission
Below 20	8	16 months
Between 20 and 30.......	26	26 months
Between 30 and 40.......	27	48 months
Between 40 and 50.......	9	36 months
Above 50	7	18 months

The average duration of symptoms in our cases before admission to the hospital was 33 months. The oldest patient in our series, L. G. (Gyn. No. 8745), came into the hospital in her fifty-seventh year, with tubercle bacilli in her urine and a history of dysuria and polyuria that had lasted for five years. The left kidney and ureter were removed and were both found to be tuberculous. Under irrigations the accompanying cystitis cleared up entirely in two years, and the patient then had five years of very good health before dying suddenly of heart trouble. The youngest patient, F. B. (Gyn. No. 23676), aged 14, had had dysuria and polyuria for eight months before coming to us. She is now well, three years after the removal of a tuberculous kidney.

Of 77 patients, 71 were white and 6 colored. On our service, one negro is admitted to the hospital for every three white women; hence it would appear that, while tuberculosis of the kidney is relatively infrequent in the colored race, it cannot be said to be rare.

Twenty-five, or 32 per cent, of the patients complained of hematuria and in eight of these it was the first symptom. Two patients first noticed general weakness, and in all the

other cases dysuria and polyuria were the first evidences of the kidney disease.

The general physical examination showed pulmonary tuberculosis in six cases. Four of these patients had active, two inactive, pulmonary tuberculosis. One patient developed tuberculous peritonitis two years after the removal of a tuberculous kidney. In four cases only was a marked enlargement of the kidney noted, and in all these cases we found at operation that we were dealing with cases of tuberculous pyelonephrosis. The routine gynecological pelvic examination showed the ureter to be thickened and tender in 23 cases. This nodular thickening with tenderness of the portion of the ureter which can be felt on vaginal examination has in this clinic been of great help in promptly directing our attention to the probability of tuberculosis of the urinary tract. The importance of ureteral palpation in women was brought out by Dr. Kelly in an article published in 1888 in Vol. VIII of the Transactions of the American Gynecological Society.

Before recording the results obtained by operative interference I shall first give a brief summary of those in patients not operated on. In five cases surgical treatment was not advised, and in two others was refused by the patients. Four of these women had tuberculosis of both kidneys, one with symptoms dating back eight years, the second two years, the third, one year, and the fourth only eight months. One of these women died in three months, the second in six months, the third in one year, and the fourth left the hospital unimproved and has not been heard of since. One woman had a unilateral renal tuberculosis with advanced pulmonary changes and in her case operation was not advised; she also left the hospital unimproved and has since not been heard of. In the two operable cases in which surgical treatment was refused the patients died within one year after leaving the hospital.

In 70 out of the 77 cases operations were performed. In 67 cases the kidney was removed and in three cases simple nephrotomies were done. The results obtained in these three last cases are very discouraging. One woman died in three months, a second in two years, and the third left the hospital unimproved and has not been heard of since. Nephrotomies were performed on these patients for the following reasons. Two of the women had bilateral renal tuberculosis with a markedly decreased urea output on both sides. When they entered the hospital, one in 1893, and the other in 1899, they were in a very bad condition and each had a unilateral pyelonephrosis. In the light of our knowledge at that time it seemed that evacuation of the pus was the only justifiable operative procedure. The third case, operated upon in 1897, was thought to be a simple pyelonephrosis, and the tuberculous nature of the kidney lesion was not recognized until material obtained at operation had been carefully studied microscopically. Unfortunately this patient later refused a nephrectomy and, after leaving the hospital against advice, died in a short time at her home.

In order to judge our ultimate results in the cases in which the kidney was removed we sent out letters to all of these patients and we now have complete records in 42 out of the 67 cases, or in 62 per cent. In all probability, of course, a number of the patients from whom we could get no record are dead, but we feel sure that our failure to trace many of them is due to the known frequency with which dispensary patients change their residence. In our series there was no instance of death under the anæsthetic. There were, however, three patients that died in less than two months, one died of post-operative pneumonia on the tenth day, the second of uremia, and the third of tuberculosis of the other kidney. These two last patients were operated upon 20 years ago and, now that our methods of studying kidney function and pathology have improved so greatly, to-day both cases would probably be considered inoperable. One woman was not at all benefited by the operation and died four years later. Four patients lived each six years after leaving the hospital, greatly improved by their operations, and then died in the seventh year. The exact cause of death in these cases is not known, but it seems probable that tuberculosis was the main factor. This means that 8, or 19.5 per cent, of the 42 women of whom we have complete records are now dead. These eight patients lived, on an average, for three years after their operation.

Two of the living patients are unimproved, one three, the other nine years after operation. Seven may be classed as greatly improved. These seven women are all able to carry on their daily occupations and complain only of slight bladder symptoms. Twenty-five, or 59.5 per cent, of these women whose present condition we now know are entirely well, with an average of 11 years since their operation. In this series we have one woman operated on 24 years ago, another 22 years ago, and two 20 years ago. These patients have been relieved of all their symptoms by operation and report themselves to be now in good general health. The following table shows graphically the above mentioned post-operative results.

Women From Whom a Tuberculous Kidney Has Been Removed and Whose Present Condition Is Known

		Per cent
Total number	42	
Known to be now dead	8	19.5
Alive and unimproved	2	4.5
Alive and improved	7	16.5
Alive and entirely well	25	59.5

We have divided all the cases in which we know the final result into two classes, one formed by those women from whom only the kidney was removed and the other by those from whom as much as the ureter as possible was removed at the same time. After careful study we have been unable to show that the removal of the ureter makes any difference in the ultimate results of the operation. Approximately 19 per cent of both groups of patients are now dead, 5 per cent are unimproved, and 76 per cent are either greatly improved or well.

Our study, however, has shown that the post-operative sinuses of the patients on whom nephro-ureterectomies were performed healed in an average time of five months, whereas the woman in whom the diseased ureters were left drained for 11 months. From this it would appear that it is better to remove the ureter along with the kidney when the condition

of the patient warrants prolonging the anæsthetic the short time necessary for the carrying out of this procedure.

CONCLUSIONS

1. In our cases of renal tuberculosis in eighteen per cent (18%) there has been a family history of tuberculosis.

2. In sixty per cent (60%) of the cases of tuberculosis of the urinary tract the lesion has been in the right kidney, in thirty-five per cent (35%) in the left kidney, and has been bilateral in four patients.

3. Renal tuberculosis occurs most frequently between the ages of twenty and forty (71%).

4. The average duration of symptoms in our women before coming to the hospital was thirty-three months

5. Renal tuberculosis in colored women, although not as frequent as in white, is by no means rare.

6. In eighty-eight per cent of our cases dysuria and polyuria were the first symptoms.

7. One-third of the women complained of hematuria on admission, and in eight patients "smoky urine" was the first sympton.

8. Thickening and tenderness of the portion of the ureter palpable on vaginal examination was present in thirty-two per cent of the patients and this sign is of great help in the early detection of cases of renal tuberculosis.

9. Of the seven patients not operated on, four are known to be dead and the other three left the hospital in a very bad condition and are probably now dead, although we have no definite information about them.

10. The three women on whom simple nephrotomies were performed all did badly.

11. The ultimate result is known in forty-two out of sixty-seven cases in which the kidney was removed, or in 62 per cent of the cases.

12. Seven of these forty-two patients may be classed as greatly improved and are now alive six years after their operations; twenty-five are entirely well with an average period of eleven years since they were discharged from the hospital. This means that 16.5 per cent of the women of whom we have records have been greatly improved by their operations and 59.5 per cent have been entirely cured.

13. Comparison of the results obtained when the ureter is removed with the kidney and when it is left *in situ* shows that, although the ultimate results are the same following the two methods, the post-operative sinus heals more rapidly when a nephro-ureterectomy is done, and this, therefore, seems to be the operation of choice when the patient's condition warrants a prolongation of the anæsthetic.

CASES OF TUBERCULOSIS OF THE KIDNEY OBSERVED IN THE GYNECOLOGICAL DEPARTMENT OF THE JOHNS HOPKINS HOSPITAL

Gynecological History number	Gynecological Pathological number	Gynecological History number	Gynecological Pathological number
4009	1017	17890	16676
4012	1018	17939	16754
4376	1180	18522	18361
5034	1670	18899	8143
5503	1902	19862	19862
		19924	19860
6035	2310	19972	19960
6074	2395	20058	20011
6609	2823	20208	19795
6912	3243	20298	No specimen
7183	3498	20847	20868
7802	4067	21625	21617
7981	4316	21813	21813
8001	4308	21859	21798
8716	5130	22010	23001
8745	5565	22040	22002
9336	5584	22849	22858
9950	6177	22311	25625
9966	6313	23366	22281
10058	6230	23464	22803
10088	6283	23518	23451
10387	6600	23549	No specimen
10809	7021	23698	23638
11255½	7275	23790	No specimen
11450	7696	24060	24025
11733	9528	24228	24178
11613	8042	24288	24318
11731	No specimen	24411	No specimen
11994	8501		
12108	8724		
12471	9192		
12866	9895		
13244	10696		
13484	10738		
13858	11535		
13951	11564		
14169	11997		
14428	12310		
14467	12203		
14549	12277		
14767	12733		
14819	12615		
14964	12834		
15085	9668		
15662	4475		
16006	14180		
16067	14461		
16144	No specimen		
16393	14838		
17217	16174		

Total 77
Number from whom kidney was removed 67
Of these 67, 42 have been heard from — 8 known to be dead. 34 known to be well.

JOHNS HOPKINS HOSPITAL BULLETIN

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PORTAL THROMBOSIS

By L. T. Webster

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Dr. William H. Welch in his classical treatise on thrombosis refers to occlusion of the portal vein as a "well characterized although usually undiagnosed affection" caused most frequently by compression of the intrahepatic branches in cirrhosis, syphilis, or tumors of the liver, by compression of the main branches or trunk by fibrous perihepatitis, chronic peritonitis, swollen lymph glands, impacted gall-stones or tumors. With pancreatic disease, gastric cancer and ulcer, is also mentioned sclerosis of the portal vein, which latter should, he said, receive more recognition.

Two years before, Spiegelberg, in a series of "Unusual Autopsy Findings," had described a case with calcification in the wall of the portal vein. Borrmann at about the same time emphasized its importance. Sachs in his comparative study of arterial and venous sclerosis considered it a manifestation of general venous involvement.

Lissauer in an excellent summary of the cases occurring at Breslau, reported in 26,687 autopsies, 68 instances of portal thrombosis which are tabulated as follows:

TABLE I

Gall-stones	9 cases.	Cancer of pancreas (primary.)	5 cases.
Syphilis of liver	7 cases.	Diseases of the spleen	5 cases.
Carcinoma of stomach with metastases to liver.	7 cases.	Cancer of pancreas (secondary.)	3 cases.
Appendicitis	6 cases.	Cancer of intestine	2 cases.
Atrophic cirrhosis	6 cases.	Cancer of liver (pr.)	2 cases.
Cancer of gall-bladder	6 cases.	Cancer of liver (sec.)	2 cases.
		Pancreatic disease	2 cases.

TABLE II.—FREQUENCY OF THROMBOSIS IN ASSOCIATED CONDITIONS

	Total number cases	Cases with portal thrombosis	Per cent cases with portal thrombus
Swelling of portal lymph glands	28	13	46%
Abscess of spleen	7	2	28%
Carcinoma of pancreas	33	8	24%
Primary cancer of liver	19	2	11%
Perityphlitis	129	6	5%
Primary carcinoma of gall-bladder	146	6	4%
Secondary carcinoma of liver	56	2	4%
Syphilis of liver	179	7	4%
Carcinoma of intestine	137	2	2%
Carcinoma of stomach with liver metastases.	503	7	1.5%
Cirrhosis of liver	711	6	.9%
Gall-stones	1407	9	.7%
Carcinoma of stomach without liver metastases.	0	0	0

Portal thrombosis is mentioned as occurring 21 times in the 6050 autopsy records of The Johns Hopkins University. It was associated with (1) cirrhosis of the liver in 7 cases, (2) with carcinoma in 6 cases, (3) with cholangitis in 4 cases and (4) with amyloid disease, (5) ulcer of the stomach, (6) Banti's disease, and (7) pylephlebitis, in one case each, respectively.

(1) *Cirrhosis of the liver*, 7 cases.

(a) Laennec: 4 cases (Cases II, V, VI, VII).
Location of thrombi.
Main portal vein and liver branches, 2 cases.
Portal vein, liver branches, splenic vein, superior mesentery, 2 cases.
Condition of vessels.
Sclerotic portal vein and branches, 4 cases.

(b) Syphilitic cirrhosis, 1 case (No. I).
Location of thrombi.
Main portal vein and liver branches.
Condition of vessels.
Sclerotic portal vein and branches.

(c) Atypical cirrhosis, 2 cases (Cases II and IV).
Location of thrombi.
Main portal vein and liver branches, 2 cases.
Condition of vessels.
Sclerotic portal vein and branches, 1 case.
No note, 1 case.

Case I.—Henrietta B. White. Autopsy 214, July 21, 1891.

Anatomical Diagnosis.—Syphilitic cirrhosis of liver, C. P. C.; Portal thrombosis (recent); acute peritonitis; colon bacillus in exudate; jaundice.

In the abdomen are about 500 cc. of turbid fluid. The peritoneal surfaces are thickened, rough. Firm adhesions bind the liver and spleen to the peritoneal wall. The splenic vein is wide, its inner circumference measuring 6 cm. There are areas of calcification and irregular thickening of the intima and here is an adherent thrombus 1 mm. in thickness, 10 mm. in length. The portal vein is also wide, its lumen is filled with a fresh thrombus adherent to the intima in spots but in general easily removed. It extends a short distance into the main branches of the portal vein. It seems to be of recent formation. The wall of the portal vein appears thickened, especially the intima.

The liver is very much deformed by coarse lobulations or nodules which result from cicatricial depressions all over its surface. These nodules are of all sizes. On section the liver is jaundiced. Fibrous bands of connective tissue extend from the capsule into the substance of the liver, producing the lobulations above described. In and about these areas is atrophy of the parenchyma. The specimen is an excellent example of syphilitic cirrhosis.

Case II.—James S. White, 41 years. Autopsy 14, 1891.

Anatomical Diagnosis.—Streptococcus septicemia following old ulcer on left leg. Double pneumonia; axillary adenitis; cirrhosis of liver; dilatation and atheroma of splenic artery and vein and of portal vein with parietal thrombosis.

The *liver* weighs 1300 grams. The surface is nodular, hob-nailed, free from adhesions. The capsule is thickened. The consistency is hard. On section the nodulated appearance is still evident. The portal, splenic, and mesenteric veins are greatly dilated. The intima is thickened with atheromatous and calcified patches, present espe-

cially in the splenic and portal vein up to the division of the portal vein into its main branches as it enters the liver. Here there are small adherent parietal thrombi. The splenic artery is extraordinarily wide and tortuous with nodular patches of thickening in the intima.

CASE III.—John S. Autopsy 462, Oct. 17, 1893.

Anatomical Diagnosis.—Diffuse suppurative cellulitis (streptococcus); lymphadenitis; ascites; chronic adhesive peritonitis; chronic interstitial hepatitis; thrombosis of portal vein.

The *liver* weighs 1700 grams. It is everywhere adherent. The capsule is thickened, fibrous in appearance and often 4 mm. in thickness. On section it appears mottled and smoky. Heavy bands of connective tissue extend inwards from the capsule. In this way areas of liver tissue are completely marked off. In the portal vein are thrombi; the main branch beginning at the junction of the principal abdominal veins is almost completely filled by a mixed thrombus which is only slightly adherent. At the entrance into the liver the vein is covered by a firmly adherent laminated thrombus, somewhat softened, which does not fill the lumen. Some of the principal branches of the portal vein in the liver are thrombosed with masses which appear to be adherent and continuous with a laminated thrombus.

CASE IV.—John M. White, 48 years. Autopsy 2706, April 13, 1906.

Anatomical Diagnosis.—Chronic splenic tumor; sclerosis of splenic and portal veins; cirrhosis of liver; thrombosis of portal branches; C. P. C. of pancreas and intestines; esophageal varices with rupture; hemorrhage into the stomach and intestine.

The splenic vein shows definite sclerosis everywhere, particularly after one leaves the hilum of the spleen. Here and there it shows definite rings of sclerosis but its lumen is everywhere patent. Such areas are particularly well marked at the branching of the vessels. At the junction of the splenic vein with the mesenteric vein is seen a large thrombus mass slightly adherent to the anterior wall of the mesenteric vein, partially occluding its lumen. The thrombus is of a grey color and shows definite ridges and furrows on its surface. The portal vein, however, shows complete occlusion of its lumen by a firm thrombus mass of greyish translucent material. In this region the lumen of the vessel is narrowed. As one goes upward one finds the two branches of the portal vein obliterated completely by a similarly appearing thrombus. In the liver some evidence of thrombosis and propagated blood clot is still evident. The aorta shows moderate arteriosclerosis.

CASE V.—William K. White, 40 years. Autopsy 4132, May 14, 1914.

Anatomical Diagnosis.—Atrophic cirrhosis of liver; portal stasis; ascites; dilated collateral circulation; organizing thrombus of portal vein and its radicals. Interstitial hyperplasia of spleen; chronic interlobular pancreatitis with hemorrhages into the islands of Langerhans; chronic interstitial nephritis; jaundice.

The splenic vein is enlarged and its lumen obliterated by an organizing thrombus. Throughout its entire extent the mesenteric vein up to the point of its larger tributaries is thrombosed even into the substance of the liver. The vessel walls are slightly thickened. The thrombus, although not very old in appearance, is firmly adherent to about half of the circumference of the vessel, and some areas appear sclerosed. The liver weighs only 810 grams. It is typically hob-nailed in appearance.

CASE VI.—John W. White, aged 53 years. Autopsy 5026, Feb. 7, 1917.

Anatomical Diagnosis.—Cirrhosis of liver; thrombosis of splenic vein; chronic splenic tumor; congestion and hemorrhage into the stomach and intestines; ascites; hydrothorax; scar on genitals; fibrous orchitis.

The liver weighs 1100 grams, and the irregular nodules would suggest the hob-nailed type of cirrhosis. The portal vein and its branches contain a thrombus, 6 cm. long, nearly obliterating the splenic vein after it has been joined by the inferior mesenteric vein but before it enters the portal vein. This thrombus is firmly adherent to one wall of the blood vessel and shows evidence of organization. The aorta shows no evidence of syphilis or of sclerotic changes.

CASE VII.—Barbara S. White, 69 years. Autopsy 5863, March 28, 1919.

Anatomical Diagnosis.—Generalized arteriosclerosis; fibrous myocarditis; cirrhosis of the liver; enlargement and fibrosis of spleen; esophageal varices; thrombosis of splenic and superior mesenteric veins; fibrinous perisplenitis; hemorrhagic infarction of jejunum with hemorrhage into stomach, duodenum, jejunum and ileum.

The liver presents the usual hob-nailed appearance of an atrophic cirrhosis. The splenic, portal, and superior mesenteric veins show marked sclerotic changes with calcification in the intima and media. A thrombus is adherent to the wall of the splenic vein and superior mesenteric veins. Propagated clots fill the entire portal system.

Summary.—In about 275 cases of cirrhosis of liver, portal thrombosis is reported in 7 cases or 2.6 per cent. Usually the main portal vein and its liver branches were implicated. In every case in which a note was made as to the condition of the veins, there occurred sclerotic changes in the intima or media. A slowing of the circulation plus an injury to the vessel wall seemed to play the all important etiological rôle.

(2) *Carcinoma,* 6 cases.

(A) Stomach, 4 cases (Cases VIII, IX, XI, XII).

Metastases to retroperitoneal glands and liver, 4 cases.

Thrombi.

1. Type:

(a) Carcinomatous thrombi,	2 cases.
(b) Blood thrombi,	1 case.
(c) Both,	1 case.
	4 cases.

2. Location:

Portal vein and liver branches,	3 cases.
Portal vein, liver branches, splenic vein,	1 case.
	4 cases.

(B) Pancreas, 2 cases (Cases X and XIII).

Metastases to retroperitoneal glands and liver, 2 cases.

Thrombi.

1. Type:

(a) Blood, 1 case.

(b) No note, 1 case.

2. Location:

Portal vein and liver branches, 1 case.

No note, 1 case.

CASE VIII.—Otto W. White, 49 years. Autopsy 175, March 4, 1891.

Anatomical Diagnosis.—Carcinoma of stomach; carcinoma of hepatic lymph glands; carcinomatous thrombus in portal vein; multiple metastases in liver.

The portal vein is of normal size. At its entrance into the liver there is projecting into it a large, smooth, carcinoma nodule. This is smooth and non-ulcerated on the surface. In the portal vein around the tumor thrombus there are soft thrombi along the wall. The veins branching near the thrombus are also filled with tumor tissue, and a large mass in the left lobe is directly continuous with the thrombus in the portal vein.

Case IX.—George C. Negro, 53 years. Autopsy 228, September 10, 1891.

Anatomical Diagnosis.—Primary carcinoma of stomach; metastases to liver, lymph glands and pleura; chronic diffuse nephritis; nodular arteriosclerosis; cysts in liver resulting from tumor communicating with portal vessels.

The liver weighs 3600 grams; it contains numerous soft nodules which vary considerably in size. Some of these nodules are very soft. On section some contain a large amount of hemorrhagic fluid which is apparently confined to the tumor mass, in cavities with smooth walls. Some of these cavities are evidently situated directly in the liver tissue. In other areas they are surrounded by a small amount of tumor tissue. In some of the larger cavities thrombi were seen projecting from the veins. Small thrombi were found in the minute branches of the portal vein. Branches of the portal vein could be traced directly into the cavities.

Case X. Barbara A. White, 51 years. Autopsy 483, January 5, 1894.

Anatomical Diagnosis.—Carcinoma of the head of the pancreas with extension into the duodenum and stomach; carcinoma of mesenteric lymph glands, retroperitoneal and gastrohepatic glands; carcinoma of the right and left adrenals, liver and lungs; thrombosis of portal vein.

The retroperitoneal glands on both sides have been converted into tumor masses which form a chain along the spine. The portal vein is thrombosed just before it enters the liver by a partially decolorized thrombus mass.

Case XI.—Frank S. White, 62 years. Autopsy 1464, January 3, 1900.

Anatomical Diagnosis.—Carcinoma of stomach with metastases to the liver, retroperitoneal and omental lymph glands and to the spleen; metastases to the hilum of liver occluding portal vein; propagated thrombus in branches of portal vein.

The splenic vein shows a dilated lumen. On dissection a metastasis is found blocking the vein completely, about 2 cm. from the junction of the splenic and portal vein, at a point where a vein enters the splenic vein from the stomach. The metastasis extends into the portal vein, obliterating its lumen completely up beyond the hilum of the liver. It is firmly attached to the vessel walls giving them a diameter of from 1 to 3 cm. and making the splenic and portal veins resemble enlarged elongated, irregular lymph nodes with metastasis. On section it is found that the centers have softened. Where this metastasis ends beyond the hilum of the liver a thrombus begins extending into the main branches of the portal vein.

Case XII.—Emma T. Colored, 32 years. Autopsy 3752, July 14 1912.

Anatomical Diagnosis. Carcinoma of the stomach with direct invasions into the retroperitoneal tissues, pancreas, transverse colon and mesenteric vessels; cancerous thrombosis of the portal vein; multiple metastases into the liver; cancerous thrombi in the pulmonary arteries; metastases to the intestine; anemia and emaciation.

The portal vein is dissected out of the tumor tissue but with the hepatic artery and the bile-ducts appears uninvolved as it enters the liver. On following the portal vein, however, into the liver for a short distance one finds a large yellowish tumor mass studded here and there with hemorrhagic zones partially occluding this vessel. Other small thrombi of a similar nature are found in the various radicals of the mesenteric veins. On section of the liver several of the portal veins are found plugged with thrombus masses similar to those described above.

Case XIII.—George T. White, 45 years. Autopsy 4635, May 25, 1915.

Anatomical Diagnosis. Carcinoma of the head of the pancreas; metastases to the liver and neighboring lymph glands; obstruction of the common bile-duct; jaundice; thrombosis of the superior mesenteric of the splenic and prostatic veins; hemorrhages in the intestinal mucosa.

Summary. In cases of carcinoma of the stomach or head of the pancreas with metastases to the liver and retro-peritoneal glands, thrombosis of the portal vein may be expected. Usually the main portal vein and its liver branches are involved. The thrombus may be carcinomatogenous or hematogenous. Pressure occlusion of the lumen and injury to the vessel wall seem to play the all important etiological rôle.

(3) *Cholangitis.*

Case XIV.—Ricky A. White woman, aged 34 years. Autopsy 1069, March 21, 1898.

Anatomical Diagnosis.—Cholelithiasis; obliteration of gall-bladder; cholangitis; general infection *(B. coli)*; acute splenic tumor; abscesses in the kidneys; thrombi in the hepatic vessels; adhesions between the gall-bladder and stomach; early peritonitis; jaundice.

Beneath the edge of the liver is a dense mass which occupies the position of the gall-bladder; about it are the adherent stomach, liver and duodenum. The bile channels are dilated and patent. In the liver the bile-ducts are dilated by a turbid fluid which runs freely from them. Thrombi firm and red are found in the portal veins and some of them are partially decolorized. The thrombosed veins are the smaller ones, usually under 0.5 cm. in diameter.

Case XV.—Richard T. White, 40 years. Autopsy 1691, March 7, 1901.

Anatomical Diagnosis.—Cholelithiasis; calculus impacted in the diverticulum of Vater, only partially filling it and occluding its duodenal orifice; acute hemorrhagic pancreatitis; fat necrosis; partial thrombosis of the splenic vein; embolism and thrombosis of branches of the portal vein.

The pancreas is represented by a swollen black tumor mass, soft and friable. The liver shows on section portal veins plugged and distended with red thrombi. When the vein is followed into one of these areas it is found to have a diameter about 4 mm.; it lies in a deep red area, and is plugged with a red thrombus which stops abruptly and is yellowish white. This may be an embolus. Occupying a portion of the lumen of the splenic vein is a mixed red and yellow thrombus mass, firm in consistency and adherent to the intima.

Case XVI.—Anthony C. White, 73 years. Autopsy 3899, April 3, 1913.

Anatomical Diagnosis.—Cholelithiasis with stones in the bile papilla and gall-bladder, subacute cholangitis; icterus; biliary cirrhosis; obstruction of the pancreatic duct; chronic and acute pancreatitis; acute suppurative portal lymphangitis with beginning pylephlebitis; liver necrosis; acute generalized peritonitis; intestinal distention; generalized arteriosclerosis; chronic diffuse nephritis; cardiac dilatation and hypertrophy.

The portal vein at the hilum shows a soft ante-mortem clot. The smaller branches of the portal vein also show many small clots.

Case XVII.—Andrew E. White, aged 58 years. Autopsy 4048, January 2, 1914.

Anatomical Diagnosis.—Operative wound for cholecystectomy and drainage of common bile-duct. Chronic cholangitis; jaundice; throm-

bosis of portal vein; surgical incision of duodenum; bilateral bronchopneumonia.

Liver. The portal vein at the hilus of the liver contains two or three friable thrombi attached to its wall. They measure about 0.5 cm. in width. The left branch is completely obliterated by a thrombus.

Summary.—In about 35 cases of cholangitis, portal thrombosis occurred in 4, or 10.5 per cent. Usually the main portal vein and its liver branches were involved. Infection of the vessel wall, although not always definitely recorded, may be assumed to play an important rôle in the etiology.

(4) *Amyloid Disease.* 1 case.

CASE XVIII.—Franklin R. White, 37 years. Autopsy 262, December 19, 1891.

Anatomical Diagnosis.—Tuberculous fibrous phthisis of both upper lobes; amyloid spleen and kidneys. Tuberculous ulceration of small and large intestine.

Liver.—Microscopical examination showed thrombi in the portal veins with extensive liver cell emboli in these vessels. Obstruction to the portal circulation was probable but no note was made regarding the condition of the vessel wall.

(5) *Ulcer of Stomach.* 1 case.

CASE XIX.—Louis F. White, 45 years. Autopsy 567, September 13, 1894.

Anatomical Diagnosis.—Perforating ulcer of the stomach; old adhesive peritonitis around the perforation; ulceration into the splenic and pyloric artery; thrombosis of the splenic artery; extension into the cœliac axis and abdominal aorta; infarction and softening of spleen; complete thrombosis of splenic vein with extension of thrombus into the portal vein; thrombosis of interlobular veins of liver.

The liver is small. In a number of the interlobular veins of the right lobe are thrombi, red, with white centers. These are continuous with a small thrombus in the portal vein. There are also a few thrombi in the interlobular veins in the left lobe, and a small thrombus enters the large branch of the portal vein running to the left lobe. The splenic vein is also thrombosed completely, the condition extending as far as the portal vein and into it, although only a small portion of the lumen of the portal vein is occupied. The splenic vein, however, is completely obliterated by a greyish thrombus adherent to its inner wall. The splenic artery is thrombosed throughout its entire extent; its origin is undoubtedly at the base of the ulcer. The thrombus extends through all the vessels of the cœliac axis into the aorta. Infection and circulatory obstruction in the splenic vein probably produced the thrombi in this case.

(6) *Banti's Disease.* 1 case.

CASE XX.—No Name. Autopsy 1398, August 7, 1899.

Anatomical Diagnosis.—Primary splenomegaly; thrombosis of splenic, portal, and mesenteric veins; anemic infarction of spleen.

The *spleen* is very soft; it weighs 2400 grams. The splenic vein is dilated to a diameter of 5 cm. and is distended with an elastic fat clot which shows points of opacity. This clot extends upward into the portal vein and even into the radicals of the portal vein. It occludes the lumen of the portal vein throughout its length. On section it is almost diffluent. The lumina of the mesenteric veins are obliterated by thrombi, largely organized.

Circulatory obstruction in the splenic vein was concerned in the formation of the thrombi in this case.

(7) *Pylephlebitis.* 1 case.

CASE XXI.—Helen C. White, 13 years. Autopsy 1409, August 24, 1899.

Anatomical Diagnosis.—Appendicitis; suppurative inflammation of a branch of the portal vein; purulent pylephlebitis; thrombosis of the portal vein; multiple abscesses of liver.

The appendix is covered with friable adhesions; it is gangrenous throughout most of its length and during separation of adhesions large rents appear in the wall. The branch of the portal vein in the mesentery which leads from the region of the appendix is distended with pus. The walls are in a large part necrotic. Traced upward this branch opens into the main portal vein behind a thrombus which is large and partially obliterating the lumen. All other branches of the portal vein are open. On section the liver, particularly the right lobe, is found to be riddled by large abscess cavities, evidently in intimate relation with the branches of the portal vein.

Infectious injury to the vessel wall and obstruction to the circulation were clearly the cause of the thrombi in this case.

CONCLUSIONS

1. In the 6050 autopsy records of The Johns Hopkins Hospital portal thrombosis has been noted 21 times.

2. It was found in 2.6 per cent of the cases of cirrhosis of the liver and was always accompanied by sclerotic changes in the walls of the portal vein or its branches.

3. Portal thrombosis was found to be common in cases of carcinoma of the stomach or pancreas which had metastasized to the liver and retro-peritoneal glands. Pressure occlusion of the lumen and injury to the vessel wall by tumor tissue were present in all the cases.

4. In 35 cases of cholangitis, portal thrombosis was noted in 10.5 per cent. Infection of the vessel wall might be inferred but was not recorded in every case.

5. One case each of amyloid disease, ulcer of the stomach, Banti's disease, and pylephlebitis were accompanied by portal thrombosis. The records, although incomplete, would lead one to infer that circulatory obstruction and infection of the vessel wall were the immediate causes of the thrombi.

6. As practical examples illustrating the combination of factors necessary to produce a thrombus, these cases are of interest.

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THE RELATION OF HISTAMIN TO LEUKOCYTOSIS

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Besides the action of histamin in producing shock, its possible relationship to some of the toxins, whose action is seen in infections and inflammatory conditions of bacterial origin, is interesting, and the possibility of its being produced in the body during these conditions seems to deserve investigation.

The question of bacterial toxins and their relationship to the systemic reactions of the body in infectious disease is an unusually complicated one, and one which is far from being clearly understood. With some organisms part of the process at least is fairly definite and we have, for example, the exotoxins of the diphtheria and tetanus bacilli and a few others. With other pathogenic organisms including pneumococci, streptococci, staphylococci, and others, the ability to produce poisons is suspected, and the profound changes in organs remote from the local infected lesions, and also changes of a general systemic nature, such as fever and leukocytosis, are supposed by many to be due to them. But the nature and character of such toxins are still obscure and although this problem has attracted a host of workers, attempts to isolate individual and specific poisons have not been successful.

Originally, Pfeiffer put forward his well-known theory of endotoxins, or poisons contained within the bacterial cell. It was assumed, that when bacteria entered the animal body and were destroyed by the action of the serum or cells, these endotoxins were liberated and poisoning resulted. But more recently the conception of endotoxins has been regarded with some uncertainty. The work of Vaughan [1] and Friedberger [2] suggests that with bacteriolysis we do not have the mere liberation of a preformed poison but the process is a chemical proteolysis produced by enzymes, by which poisonous groups of the bacterial protein-molecule are set free. These toxic cleavage products are the result of the reaction between blood plasma and the bacterial cell; they are not specific and may be formed from all bacterial proteins pathogenic and nonpathogenic.

Another view of the bacterial toxins, of quite a different nature, is that of Jobling and Petersen,[3] who believed that in infectious processes most of the toxic substances originate from serum proteins, and the bacteria act merely by removing the antiferments from the serum, thereby setting free the normal serum ferments and permitting them to act upon the serum proteins.

These views do not bring us to any definite conclusions as regards the type of toxins with which we are dealing. Chemically, they are as yet undefined, for we have no knowledge of their constitution, except in so far as it has been hitherto impossible to separate them from the protein molecule. Their relation, then, to split protein products is accepted. Vaughan [1] states that, although we do not know the chemical structure of the protein poison, we are certain that it is not an amino-acid, although it may be closely related to one of these; and it will probably be found that the protein molecule contains a whole spectrum of poisons with slight variations of structure. He also mentions the fact that in its action the protein poison seems quite similar, if not identical, with the histamin of Barger and Dale.

It seems important, therefore, to test the effect of repeated small doses of this substance upon experimental animals. If it represents one of the actual protein poisons which are produced during an infection, one might expect that such injections could give rise to some of the systemic signs which we are accustomed to see in infections, such as fever and leukocytosis. With this intention some experiments were carried out.

Fever has been produced experimentally by the parenteral administration of proteins of diverse origin (Vaughan, Wheeler, and Gidley)[4] and these workers have found that by modifying the dose the type of fever could be determined at will. Also, changes in the blood picture, such as the production of an eosinophilia, have been accomplished by Schlecht,[5] who found that on repeated injections of protein into a sensitized animal the eosinophiles were increased, and his results were also borne out by Chancellor,[6] who found that an increase of the same element of blood cells could be produced after injections of protein poison. Neither of these authors report any striking increase in the total number of white cells circulating in the blood stream during the course of their experiments.

The experiments described below were undertaken in an effort to determine what influence histamin, when introduced parenterally into experimental animals, would have on the number of white blood cells circulating in the blood stream and what variations in temperature it could produce.

Solutions, made up in small quantities of distilled water, of commercial preparations of histamin hydrochloride (Hoffman and La Roche Chemical Works) were employed for the injections.

Rabbits, although described by Dale as being relatively refractory to the effects of histamin, were employed for this work, owing to the facility with which blood for counting purposes can be drawn from the ear veins. It is worth mentioning here, however, that the greatest care was necessary in order to secure accurate white blood counts. Counts from the ear veins of animals, from which the blood was pressed or milked, proved inaccurate, showing an erroneous increase in the number of white blood cells. Constant exposure of the ear vein to manipulation, and cutting of the ear vessels producing localized phlebitis, some oedema and often inflammation, rendered counts inaccurate. Apparently the best results were obtained from free flowing arterial blood.

Intravenous Injections.—Primarily single large doses (1-1.5 mg. per kilo) were introduced into the rabbit's ear vein. As a rule, this dose was sufficient to produce violent symptoms. These came on in a few (10 to 20) seconds, with primary dyspnœa, then marked unsteadiness of movements followed by severe clonic convulsions, subsequently to which the animal lay motionless with head drawn back and limbs outstretched showing shallow and almost imperceptible respirations. After this there was a period in which the animal showed symptoms of shock, the extremities were cold and the blood pressure apparently low, the flow of blood from the ear vein was sluggish and its color dark.

Leukocyte counts were made at hourly intervals following this injection over a period of from four to six hours and again one count on the next day. No striking changes were noted in the number of white blood cells in the blood stream in these experiments.

Again smaller doses (0.25-0.5 mg. per kilo) were administered intravenously at hourly intervals over a period of from four to six hours. These individual doses were not sufficient to produce violent symptoms and generally the animals showed little more than transient dyspnœa following the injection. White blood counts, taken in the same fashion as above, again showed no variations.

Subcutaneous Injections.—About 15 experiments of this type were tried. It was thought that by subcutaneous injection larger doses could be given, the rate of absorption would be slower, and the effects prolonged. Doses of from 14 to 16 mg. per kilo were employed, injections being made in the region of the back. The rabbits survived large doses, 25 mg. being administered at one time to one rabbit. Within from two to five minutes after a small subcutaneous injection the animal generally showed signs of uneasiness, restlessness and often a mild diarrhoea. Following this the animal became stuporous, remaining in this condition for a period of from one to three hours with subsequent recovery which was apparently complete. During the first hour following the injection there was definite shock. The extremities were cold and marked difficulty in dilating the vessels of the ear vein was generally experienced. The blood pressure was apparently low, the flow of blood sluggish and its color dark.

In all experiments almost hourly leucocyte counts were made following the injection. The results of the counts showed little change in the number of white blood cells during the first two hours, with occasionally a slight leukopenia, but following this the counts seemed to show a constant rise, occasionally as much as an increase of eight to ten thousand cells per cubic millimeter and a return to normal in from five to six hours. This transient leukocytosis, however, did not seem to exceed physiological limits, as almost similar changes were noted on several occasions after the rabbits had been eating.

One experiment was performed to test the cumulative action of histamin and two subcutaneous doses were administered, the second one being given three and one-half hours after the first, at a period when the white blood cells were increased. A sharp drop in the number of white blood cells and a gradual return to normal with no subsequent leukocytosis were observed.

One rabbit received nine subcutaneous injections during a period of five weeks. The white blood cells always returned approximately to the same constant number during the days between experiments.

In order to observe the possible variations of temperature which histamin injections might produce, a number of experiments were tried on guinea-pigs. Primary single doses (3-5 mg. per kilo) were administered intraperitoneally. Following such an injection the guinea-pigs almost invariably showed signs of respiratory difficulty. There was often also weakness on the part of the animals and inability to stand. Generally, however, recovery was apparently complete. The temperature, taken per rectum, showed, as a rule, a fall of one or two degrees during this period of shock, but a return to normal was established in an hour's time.

CONCLUSIONS

1. Following intravenous injections of histamin hydrochloride into rabbits, no material change in the number of white blood cells circulating in the blood stream was observed.
2. Larger doses given subcutaneously were followed by a slight leukocytosis coming on about two hours after the injection and lasting from five to six hours with a return to normal.
3. This rise in the white blood count was not dependent on the size of the injected dose and did not seem to exceed physiological limits.
4. Repeated subcutaneous injections over a period of six weeks did not cause any change in the number of circulating white blood cells.

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THE INFLUENCE OF THE ANTERIOR LOBE OF THE HYPOPHYSIS UPON THE DEVELOPMENT OF THE ALBINO RAT

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and

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Introduction.—It was thought of interest, in the course of a study on the relation of the thymus gland to growth, to compare the changes that might be produced by feeding animals with this gland with those changes brought about by feeding animals with the anterior lobe of the hypophysis. Robertson,[1, 2] and Goetsch[3] had described in detail definite changes in the normal development of animals after feeding hypophysis. We, therefore, fed two rats of a litter of 8 with the desiccated substance of the anterior lobe of the hypophysis. The other rats of the litter received either thymus or the routine diet. The results of this preliminary experiment were negative. We, therefore, determined to carry on further studies by feeding rats the anterior lobe of the hypophysis gland, using thymus-fed rats and those receiving no gland as controls, with the hope of learning more exactly the relation that the hypophysis bears to early developmental changes.

Experimental Method.—The method employed in this study consists in the oral administration of the desiccated powdered gland.† It is prepared by drying in a vacuum at 35° C. the anterior lobe of the hypophyses of young calves. This is then extracted with ether and the dried product carefully powdered. One gram of the desiccated powder represents 4.5 grams of the fresh gland. As the experiments were in progress over a period of three years, three lots of this powder were used. The dosage varied from 0.07 gm., the amount usually given by other workers, to 0.3 gm. daily. The powder was mixed with small amounts of the diet and fed in small dishes placed in the compartments of the cage. The rats were kept confined until all the powder was eaten. In the early experiments, the animals were often deprived of other food for a time in order to insure more rapid and complete ingestion of the powder. Control and fed animals were treated alike.

The animal selected for these feeding experiments was the albino rat (Mus norvegicus albinus) standard stock of the Wistar Colony, Philadelphia. The data on the development of the albino rat, as compiled by Donaldson,[4] were freely used for purpose of comparison. The animals were fed with the gland from the third week, when the rats were weaned, for various lengths of time up to ten weeks. They were kept in specially constructed cages, which afforded protection from light and ample room for their normal activities. Precautions were taken to keep the rats under the most favorable surroundings for their development. The diet consisted of wheat or corn bread and milk, together with mixed grains or specially purified food products. The first six litters received liberal amounts of bread and milk twice daily with additional feedings of the mixed grains. A similar diet was given to litters seven, eight, and nine, except that each rat received an equal amount of whole milk, the amount varying from 4 to 12 c. c. according to the size of the animal. Litter 10 received a diet recommended by McCollum, consisting of 20 per cent purified casein, 2 per cent agar, 72.3 per cent dextrin, 2 per cent butter or egg-yolk and 3.7 per cent of a salt mixture made up as follows:

NaCl	0.173
$NaH_2PO_4H_2O$	0.347
K_2HPO_4	0.954
Ca lactate	1.3
Fe lactate	1.18

The rats were killed with ether and accurate measurements of body and tail-length were then made. The testes with the epididymes were weighed. In two experiments single testes of control and fed animals were surgically removed for microscopical studies after a few weeks of feeding with the gland. The remaining testes of these animals were studied at the close of the experiment. The organs of reproduction of all the animals were fixed in Zenker's neutral formalin and stained with hematoxylin and eosin for microscopical study.

Results.—The results of the experiments include the observations of the activities, gross developmental changes, studies of the body and testicular weights, body and tail measurements and microscopical studies of the reproductive and endocrine glands.

The activities of all the rats, such as playfulness, sexual proclivities, appetite and sleep were normal in all instances. It was not noted that the largest doses of hypophysis gland affected the activities or the nutrition. The gland produced no toxic symptoms, such as diarrhœa, muscular contractions or emaciation.

No differences in gross developmental changes, such as, size, color, or texture of fur, were noted between the hypophysis-fed animals and those receiving no gland. The testes descended almost synchronously. The consistency and size of the testes showed no variation. The thymus-fed rats, on the other hand, were in most instances larger, better nourished, their fur was sleeker, thicker and had a more yellow tinge. The testes of these animals descended earlier and in most instances were larger than those other members of their respective litters. These differences were much less marked in

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† The powder was kindly supplied to us by Armour & Co.

the latter experiments where the diet was most carefully controlled.

Variation in the appearance of the mammae was slight during the early development of the rat and the time of the rupture of the hymen was found to be inconstant and not definitely related to conception.

A comparison of the measurements of the body and tail lengths of the hypophysis-fed and control rats shows inconstant and very slight differences.

The weights of animals in each litter are recorded in the accompanying tables and curves. Detailed comparisons between the weights of the hypophysis-fed and the control animals have been made in various ways.

First, the average weight of the hypophysis-fed, male and female, animals at death, has been compared with a similar average weight of their respective controls receiving no gland substance or gland other than hypophysis.

(1) Hypophysis-fed, male, 91 gms. Control, no gland, male, 88.8 gms.
Hypophysis-fed, female, 86.4 gms. Control, no gland, female, 84.2 gms.

(2) Hypophysis-fed, male, 72.2 gms. Thymus-fed, male, 77.0 gms.
Hypophysis-fed, female, 70.4 gms. Thymus-fed, female, 76.8 gms.

These figures show an increase of 2.9 per cent and 2.6 per cent more in the weight of the hypophysis-fed animals, male and female, respectively, than the controls receiving no gland, and an increase of 6.6 per cent and 9.0 per cent of the thymus-fed animals more than the hypophysis-fed animals.

Secondly, a comparison of the total percentage of weight gained by the hypophysis-fed rats and their controls has been made.

(1) Hypophysis-fed, male, 411.8%. Control, no gland, male, 401.8%.
Hypophysis-fed, female, 424.0%. Control, no gland, female, 401.2%.

(2) Hypophysis-fed, male, 299.6%. Thymus-fed, male, 333.1%.
Hypophysis-fed, female, 314.2%. Thymus-fed, female, 331.0%.

These figures show that the hypophysis-fed animals, male and female, gained 2.5 per cent and 5.6 per cent respectively, more than the controls receiving no gland, and further that hypophysis-fed male and female rats gained 11.1 per cent and 5.0 per cent respectively less than thymus-fed controls.

Thirdly, a comparison was made of the average actual weight of the hypophysis-fed rats with their controls at various periods in their development. The average weight of male and female rats fed hypophysis gland for 28-30 days was compared with the average weights of their controls.

(1) Hypophysis-fed, male, 269.4 gms. Control, no gland, male, 273.8 gms.
Hypophysis-fed, female, 279.1 gms. Control, no gland, female, 272 gms.

(2) Hypophysis-fed, male, 199.0 gms. Thymus-fed, male, 222.4 gms.
Hypophysis-fed, female, 191.3 gms. Thymus-fed, female, 218.0 gms.

These figures show that the hypophysis-fed male animals gained 1.6 per cent less than the rats receiving no gland, and the females 2.5 per cent more; also that the hypophysis-fed males gained 10.5 per cent less and the females 12.3 per cent less than the thymus-fed control during the corresponding period.

Finally, a comparison was made of the average testicular weights, both actual and their ratio to the body weight, at death and at periods during the experiments.

Hypophysis-fed, 1.618 gms. (actual weights) Hypophysis-fed, 1.002 gms.
Control, no gland, 1.555 gms. (actual weights) Thymus-fed, 1.666 gms.
Hypophysis-fed, 1.565 gms. (relative weights) Hypophysis-fed, 1.635 gms.
Control, no gland, 1.527 gms. (relative weights) Thymus-fed, 1.65 gms.

These figures show that the actual weight of the testes of the hypophysis-fed rats is 4 per cent more than that of the control receiving no gland, and 14.3 per cent less than of the thymus-fed animals, also that the relative weight of the testes of the hypophysis-fed animals is 2.5 per cent more than that of their controls receiving no gland and 0.9 per cent less than that of the weight of the thymus-fed rats.

Comparisons of testicular weights have been made from Experiments VIII and IX, in which one testis was surgically removed from both hypophysis and control animals some time before the animals were finally killed.

EXPERIMENT VIII

(1) WEIGHTS OF TESTES AFTER 25 DAYS OF OBSERVATION. (2) WEIGHTS AFTER 40 DAYS

	Actual weights			Relative weights	
(1)	Hypophysis-fed	0.357 gms.	(1)	Hypophysis-fed	0.826%
	Control (no gland)	0.385 gms.		Control (no gland)	0.908%
	Thymus-fed	0.421 gms.		Thymus-fed	0.888%
(2)	Hypophysis-fed	0.732 gms.	(2)	Hypophysis-fed	1.1%
	Control (no gland)	0.855 gms.		Control (no gland)	1.2%
	Thymus-fed	0.842 gms.		Thymus-fed	1.2%

EXPERIMENT IX

(1) WEIGHTS OF TESTES AFTER 33 DAYS OF OBSERVATION. (2) WEIGHTS AFTER 50 DAYS

	Actual weights			Relative weights	
(1)	Hypophysis-fed	0.54 gms.	(2)	Hypophysis-fed	1.02%
	Control (no gland)	0.33 gms.		Control (no gland)	0.762%
	Thymus-fed	0.51 gms.		Thymus-fed	0.98%
(1)	Hypophysis-fed	0.933 gms.	(2)	Hypophysis-fed	1.1%
	Control (no gland)	0.735 gms.		Control (no gland)	1.15%
	Thymus-fed	0.87 gms.		Thymus-fed	1.1%

These figures show that the actual weight of the testis of the hypophysis-fed rat in Experiment VIII after 25 days of feeding is 7.8 per cent smaller than the control receiving no gland and 17.9 per cent smaller than the thymus-fed control. After 40 days of observation the remaining testis of the hypophysis-fed animal is 3.2 per cent and 15 per cent, respectively, smaller than the controls. A similar comparison of the relative weight shows the testes of the hypophysis-fed rat to be 9.68 per cent and 7.5 per cent respectively smaller than the controls.

In Experiment IX the testis of the hypophysis-fed rat is 36 per cent heavier in actual weight than that of the control receiving no gland and 5.8 per cent heavier than the thymus-fed control. A corresponding comparison of relative weights shows that in the controls the testes are 25 per cent and 4 per cent respectively lighter than in the hypophysis-fed rat. Seventeen days later it will be noted that there is no difference in the relative weights of the hypophysis-fed and thymus-fed rats and that now the testis of the control rat, receiving no gland, is 0.5 per cent heavier than either.

Microscopical sections were made of the reproductive organs and endocrine glands of eight litters of rats. No differences could be detected between the sections from the hypophysis-fed and their respective controls. The testes of rats from litters 8 and 9, when seven and one-half weeks of age, were removed surgically. The tubules of the testes were completely filled with spermatogonia, spermatocytes and a few spermatids. The findings in the hypophysis-fed and the controls were identical. No differences were noted in the sections of the testes of these animals later when they were removed at death.

Discussion.—The interpretation of some of the results, such as, the activities of the animals, admits little opportunity for discussion, as the animals showed no departure from the normal. The only variation in development between the hypophysis-fed and control animals was the accelerated growth of the thymus-fed rats. The significance of this will be discussed in a later publication. The data presented in the weight tables and curves and the microscopical findings of the endocrine glands give the most satisfactory method to determine any developmental variation. It will be noted that the weight curves show a surprising similarity throughout all the experiments. This is especially well illustrated in Experiment X, in which the diet was most carefully regulated—each animal receiving approximately equal amounts of food accessories and proteins. Occasional exceptions are encountered as in litter 4. In this instance members of the litters had snuffles and the results were not included. In litter 9 the male control was much smaller than any of the others in the litter. This animal always appeared well and undoubtedly represented a variation of the species. Although the total percentage of gain of all the hypophysis-fed male and female rats was 2.5 per cent and 5.6 per cent, respectively, greater than that of the controls receiving no gland, this difference does not justify the conclusion that it was caused by a specific principle in the hypophysis gland. The difference can easily be accounted for by the normal variation in the species. Robertson [1, 2] has referred to certain phases of accelerated growth due to feeding the anterior lobe of the hypophysis. This did not exist in our experiments as can be seen from the weight curves. In a period of observation of four weeks the male hypophysis-fed animals gained altogether 1.5 per cent less than the controls.

Special attention was given to the variation in the weights of the testes which has been noted after feeding the anterior lobe of the hypophysis. Almost identical results were obtained as in the comparative studies of body weight. The testes of the hypophysis-fed rats weighed 2.5 per cent more than those of the controls receiving no gland and 0.9 per cent less than those of the thymus-fed. The results of the weights of the testes surgically removed seem somewhat contradictory, but must be interpreted as showing no effect of the feeding. The discrepancy in the findings is explained on the basis that the control rat was undersized, and the weight of the hypophysis-fed animal was normal. This view is confirmed by comparison with normal weight curves and by the failure to note differences in the microscopical sections of the testes.

The results of our series of experiments are in agreement with the findings of Gudernatsch,[6] Lewis and Miller [7] and Hoskins.[8] Clark,[9] Robertson,[1, 2] and Goetsch [3] have concluded that after feeding young animals with the anterior lobe of the hypophysis growth was accelerated. Others, notably Pearl,[10] and Wulzen [11] considered that a retardation of growth followed the administration of the gland. These contradictory results may be explained by a failure to take into consideration certain factors which in themselves may cause the changes thought to be referable to the hypophysis. It is notable, for instance, that many observers have given little consideration to the normal variation in development of the particular species of animal utilized for the experiments. Hoskins [8] especially emphasized this point. Goetsch [3] in his experiments used a so-called "narrow-selection" race of rats whose normal development showed marked variation as shown by the varying weights of his control. His animals were bred primarily for a constant color. The albino rat (mus norwegicus albinus) used by us, and also by Hoskins [8] is peculiarly adapted for feeding experiments such as we have reported. This animal has been carefully "standardized" by breeding and the coefficient of variation of the individual rat has been compiled by Donaldson.[4]

Another factor accountable in part for the conflicting results above noted is the use of too limited a number of animals. Goetsch, for instance, who reports that the anterior lobe of the hypophysis stimulates growth and sexual development refers in his article to only six animals that were fed with the anterior lobe of the hypophysis. Our results are based on a series of 68 animals, about 30 of which were fed the gland substance while the others were used for controls.

We are led to believe, especially from experiments still unpublished, that careful regulation of the diet is an essential factor in experiments such as have been reported, and undoubtedly many of the discrepancies in results can be attributed to the administration of unequal amounts of egg, cream and similar articles of food. The work of Osborne and Mendel [12] and of McCollum has emphasized the important relation to growth of food accessories and adequate amounts of ingredients, such as protein and proper salt mixtures. These authors have shown that growth is induced by the so-called fat-soluble A and water-soluble B accessories such as are found in large quantities in egg-yolk and milk. Essentially no consideration has been taken of these factors in past feeding experiments.

Other factors, such as the general health of the animals may explain the discrepancies in the results referred to in the

literature. Snuffles is undoubtedly the commonest cause for variation in the weight of albino rats.

An interpretation of the results of our experiments, as they may add to the knowledge of the function of the hypophysis, demands finally a consideration of the value of the method of experimentation. Although it is recognized that the secretion of the endocrine glands normally reaches the body fluids by means of the circulation and that digestive processes may produce fundamental changes in the substance given by mouth, this method seems to be the one of choice.

Summary.—The desiccated powder of the anterior lobe of the hypophysis gland of young calves has been fed to albino rats of a standard stock. The experiments were begun when the animals were three weeks old and lasted for periods of from seven to ten weeks. Sixty-eight animals were used for the experiments, about half of this number being controls. These animals were observed for differences in activity, in the condition of their fur, in their nutrition and in their skeletal development. Special emphasis has been laid upon differences in external sexual characteristics, changes in body weights and differences in the microscopical findings of the reproductive organs and the endocrine glands. Dietary precautions were taken in order to differentiate the effect of food and the gland substance. The hypophysis-fed animals developed normally and showed no differences beyond the variations of their species. The autopsy studies also show no differences.

Conclusion.—Feeding of the desiccated powder of the anterior lobe of the hypophysis of calves to albino rats from three to ten weeks of age in doses of 0.04 to 0.3 of a gram causes no change in their normal development.

TABLE I

(Litter 1.)

Born Apr. 29. 1916. (7 animals.)
Feedings begun May 25. 1916. 26 days old.
Animals killed June 24. 1916. 56 " "
Duration of experiment, 30 days.

Sex	Feeding	Wght. May 25	Dose	Wght. June 3	Wght. June 9	Dose	Wght. June 17	Wght. June 24	Percentage wght. gained
M.	Hypophysis...	19.5	0.04	24.5	45.	0.1	53.	64	228
F.	Hypophysis...	16.5	0.04	22.	42.	0.1	51.	59	257
M.	Control.......	18.	0	27.5	44.	0	54.5	66	266
F.	Control...	17.	0	26.5	42.	0	50.	62	265
M.	Thymus.....	18.5	0.2	29.	54.	0.3	67.	75	305
M.	Thymus.......	17.	0.2	23.5	44.5	0.3	57.	68	300
F.	Thymus.......	16.	0.2	21.	39	0.3	50.	63	294

Notes.—No differences were noted at any time either in activities or in external characteristics. The testes of the hypophysis-fed animals descended about the same time as did those of the control animals. Although the weight curve of this litter is below the normal curve (Donaldson) there was no evidence of snuffles. The animals took the gland rather poorly and it was often necessary to limit the amount of food given.

TABLE II

(Litter 2)

Born Apr. 28, 1916. (8 animals.)
Feedings begun May 25, 1916. 27 days old.
Animals killed June 24, 1916. 57 " "
Duration of experiment, 30 days.

Sex	Feeding	Wght. May 25	Dose	Wght. June 3	Wght. June 9	June 9, dose	Wght. June 17	June 21, dose	Wght. June 24	Percentage wght. gained
M.	Thymus.......	21.	0.2	32.5	59	0.3	73	0.4	76.	261.9
F.	Thymus.......	21.	0.2	33.	58	0.3	65	0.4	70.5	231.2
M.	Hypophysis...	20.	0.04	28.	49	0.1	65	0.2	69.	245.0
F.	Hypophysis...	20.	0.04	28.5	51	0.1	65	0.2	57.5	187.5
M.	Control.......	22 5	0	29.	51	0	62	0	66.5	195.5
F.	Control... ..	19.5	0	25.	44	0	51	0	52.5	169.2
M.	Lymph........	21.	0.2	30.5	59	0.3	80	0.4	84.5	302.3
F.	Lymph........	20.	0.2	28.	55	0.3	60	0.4	72.	260.0

Notes.—The fur of the thymus-fed animals is distinctly more yellow and has a sleeker appearance than that of the controls; the latter is whiter, thinner and less coarse. These animals together with the lymph gland-fed animals are distinctly larger in every way than the controls. The hypophysis-fed animals show no difference in size from the controls. The hymen of the lymph gland-female is ruptured, the others are intact. The testes of the thymus and lymph gland-fed animals are distinctly larger than the controls. There is no appreciable difference between the size of the testes of the hypophysis-fed animals and those of the controls.

Microscopic Findings.—Testes: (Hypophysis-fed rats.) The tubules are completely filled with cells consisting of spermatogenia, spermatocytes in all stages and in some instances spermatids. The control animal shows similar findings to that noted in the hypophysis-fed.

Ovaries: (Hypophysis-fed rats.) These show evidence of active ovulation, many Graafian follicles almost fully developed but no corpora lutea. The control animal shows similar findings.

Uterus: (Hypophysis-fed rats.) The uterine mucosa is rather flat, shows no evidence of hyperplasia and there are a few glands in the endometrium. The control animal shows similar findings.

TABLE III

(Litter 2)

Table of Measurements of Weight of Testes, Litter 2

Sex	Feeding	Body length	Weight testis	Ratio of testis to body weight
M.	Thymus.....................	26.9	1.18	1.55
F.	Thymus.........	26.7	....	....
M.	Hypophysis..........	26.3	1.2	1.74
F.	Hypophysis.	26.6	....	....
M.	Control.....................	26.3	1.08	1.62
F.	Control...........	23.8	....	....
M.	Lymph...................	27.8	2.01	2.38
F.	Lymph...........	27.3	....	....

TABLE IV

(LITTER 3)

Born June 17, 1916. (Ten animals.)
Feedings begun July 7, 1916. 20 days old.
Animals killed July 24, 1916. 37 " "
Duration of experiment, 17 days.

Sex	Feeding	Dosage	Wght. July 7	Wght. July 13	Wght. July 20	Wght. July 24	Percentage wght. gained
M.	Nucleic acid......	0.0	15.	20.5	29	32	113
F.	Nucleic acid.....	0.02	12.5	19.5	22	31	148
M.	Thymus...........	0.1	15.	28.	41	51	240
F.	Thymus...........	0.1	12.5	25.5	42	47	276
M.	Hypophysis.......	0.05	16.	25.	37	44	175
M.	Hypophysis.......	0.05	14.	23.	36	44	215
M.	Spleen............	0.1	15.5	23.	37	43	214
M.	Spleen............	0.1	15.5	25.	42	51	229
M.	Control...........	0	13.5	24.	33	39	188
F.	Control...........	0	13.	22	32	36	177

TABLE V

(LITTER 3)

Sex	Feeding	Body length	Weight of testes	Ratios of testes to body weight
M.	Nucleic acid............	19.1	0.35	1.09
F.	Nucleic acid.......	19.	....	....
M.	Thymus....................	22.	0.65	1.27
F.	Thymus....................	21.5	....	....
M.	Hypophysis................	21 2	0.42	0.95
M.	Hypophysis................	21 1	0.45	1.02
M.	Spleen......	20.5	0 50	1.17
M.	Spleen......................	20.9	0.7	1.37
M.	Control.....	20.5	0.5	1.28
F.	Control............	20.1	....	....

July 13: Animals taking gland well, all in excellent condition, no snuffles.

July 23: For the past ten days with the exception of hypophysis-fed animal, the glands have been taken poorly. It has been necessary to feed the glands twice a day and to starve the animals for a few hours to insure that all the gland is eaten. The control animals are fed in the same manner. The first spleen-fed rat takes its gland poorly. The thymus-fed animals are larger than any of the others. The testes of these animals are distinctly larger, the scrotum redder and the testes more fully descended. There is no notable difference in general appearance or in the external genitalia between the pituitary-fed and the control animals.

Microscopic Studies.—The testes of the hypophysis-fed rats consist of tubules compactly filled with spermatogonia, spermatocytes and spermatids. No fully developed spermatozoa are seen. The section of epididymes shows columnar epithelium almost filling the lumen. Sections of testes and epididymes from control animals show almost identically the same findings.

TABLE VI

(LITTER 4)

Born Dec. 18, 1916. (Eight animals.)
Feedings begun Jan. 9, 1917. 21 days old.
Animals killed Feb. 20, 1917. 63 " "
Duration of experiment, 42 days.

Sex	Feeding	Dose *	Wght. Jan. 9	Wght. Jan. 13	Wght. Jan. 20	Wght. Jan. 27	Wght. Feb. 3	Wght. Feb. 10	Wght. Feb. 20	Percentage gained
M.	Hypophysis...	0.05	19.	22.7	35.25	56.5	67.	86	116.	510.
F.	Hypophysis...	0.05	16.5	19.	31.5	47.5	56.5	67	86.	434.
M.	Control.......	0	19.	23.	32.5	46.	61.	77	93.5	391.
F.	Control.......	0	19.	22.7	33.75	44.	56.	70	87.5	360.
M.	Hypophysis...	0.05	17.	21.	32.5	57.25	67.	84	114.	573.5
F.	Hypophysis...	0.05	18.	18.	27.	42.5	53.	59	77.5	330.
M.	Control.......	0	19.5	23.5	33.75	45.	60.	74	98.5	405.
M.	Control.......	0	19.	23.	32.	44.5	60.	74	98.5	418.4

* Hypophysis given in divided doses.

NOTES.—Soon after the beginning of this experiment certain rats, especially the hypophysis-fed females, developed snuffles. All the animals were isolated and the results of this litter were not included. The table of weights and weight curves illustrate the effect of a mild attack of snuffles upon growth.

TABLE VII

(LITTER 5)

Born Dec. 25, 1916. (Six animals.)
Feedings begun Jan. 13, 1917. 19 days old.
Animals killed Feb. 28, 1917. 65 " "
Duration of experiment, 46 days.

Sex	Feeding	Dose	Wght. Jan. 13	Dose Jan. 16.	Wght. Jan. 20	Wght. Jan. 27	Wght. Feb. 3	Wght. Feb 10	Wght. Feb. 17	Wght. Feb. 24	Wght. Feb. 27	Percentage wght. gained
M.	Control.......	0	14.7	0	29.	43.	59.	75.	97	119.5	125.	750.3
F.	Control.......	0	14.	0	31.	47.	63.	75.5	93	107.5	108.	671.4
M.	Hypophysis...	0.05	14.	0.75	25.5	32.5	47.	60.	84	103.5	119.	750.
F.	Hypophysis...	0.05	14.	0.75	29.	42.5	57.	61.	82	94.5	105.5	654.
M.	Hypophysis...	0.05	13.	0.75	28.	42.2	57.	70.	97	116.	124.	853.8
F.	Hypophysis...	0.05	11 5	0.75	25.	37.	46.5	58.	77	91.	93.	708.

TABLE VIII

(LITTER 5)

Sex	Feeding	Tail Length	Body Length	Weight of Testes	Ratio of testes to body weight
M	Control.....	15.6	17.9	2.902	1.62
F.	Control................ ..	15.	16.5	....	....
M.	Hypophysis.......	15.2	17.2	1.36	.79
F.	Hypophysis........... ...	15.4	15.9	...	....
M.	Hypophysis.	15.3	17.5	2.1	1.69
F.	Hypophysis..............	14.8	15.7	....	....

NOTES.—*Jan. 27.*—Rats in excellent condition, activity normal. Testes fully descended; no differences in size.

Feb. 10. The hypophysis-fed are somewhat smaller than the control rats. There is no difference in size of testes or condition of fur.

Feb. 24. Animals in excellent condition. No differences in activity. Fur and external genitalia of hypophysis-fed and control animals show no differences.

Microscopic Findings.—Sections of ovaries, Fallopian tubes and testes of control and hypophysis-fed animals show no differences. The testes are fully developed and the ovaries show numerous corpora lutea.

TABLE IX

(LITTER 6)

Born Dec. 27, 1916. (Six animals.)
Feedings begun Jan. 18, 1917. 22 days old.
Animals killed Feb. 28, 1917. 63 " "
Duration of experiment, 41 days.

Sex	Feeding	Dose	Wght. Jan. 18	Wght. Jan. 27	Wght. Feb. 3	Wght. Feb. 10	Wght. Feb. 17	Wght. Feb. 24	Wght. Feb. 27	Percentage wght. gained
M.	Hypophysis...	0.05	24.5	47.5	71.5	90.5	116	139.5	152.	520
F.	Hypophysis...	0.05	25.5	51.	69.	82.5	109	123.	135.	430
M.	Control.......	0	24.5	47.7	63.	81.	105	134.5	140.5	472
F.	Control.......	0	23.5	47.5	65.5	84.	101	109.5	116.	395
M.	Hypophysis...	0.05	23.	48.	69.5	90.	120	142.	154.	570
F.	Hypophysis...	0.05	23.5	47.5	74.	91.	123	132.5	130.	453

TABLE X

(LITTER 6)

Sex	Feeding	Tail length	Body length	Testes	Ratio of testes to body weight
M.	Hypophysis...............	15.8	17.2	2.7	1.77
F.	Hypophysis...............	15.6	16.7	....	....
M.	Control....................	14.9	17.5	2.35	1.67
F.	Control....................	15.4	15.8	...	....
M.	Hypophysis..........	15.6	17.9	2.43	1.57
F.	Hypophysis...............	14.9	16.	....	....

NOTES.—*Jan. 27, 1917.* All the animals are in excellent condition. The testes of the hypophysis-fed and control animals descended almost simultaneously. They show no difference in size.

Feb. 10, 1917. The male hypophysis-fed rats are larger than the control. There is no definite difference in the state of nutrition or appearance of their coats.

Feb. 27, 1917. All the rats are in excellent condition. Their activities are normal. No precocities are noted. No appreciable variation in appearance of external genitalia is noted.

Microscopic Findings: Testes. (Hypophysis-fed.) Lumina of tubules are filled with mature spermatozoa. There is evidence of active spermatogenesis. Control: Findings are as noted for the hypophysis-fed rats.

TABLE XI

(LITTER 7)

Born Dec. 27, 1916. (Ten animals.)
Feedings begun Jan. 20, 1917. 24 days old.
Animals killed Feb. 27, 1917. 62 " "
Duration of experiment, 38 days.

Sex	Feeding	Dose	Wght. Jan. 18	Wght. Jan. 27	Wght. Feb. 3	Wght. Feb. 10	Wght. Feb. 17	Wght. Feb. 24	Wght. Feb. 27	Percentage wght. gained
M.	Control...........	0	15.2	38.	59.	76.	105.	130.	136.5	798
F.	Control...........	0	15.	35.5	54.5	71.	94.	115.	122.	715
M.	Hypophysis.......	0.05	17.	35.	49.	63.	85.	107.	112.5	562
F.	Hypophysis.......	0.05	15.5	35.	54.	66.	90.5	109.5	116.	649
M.	Control........	0	15 5	34.5	52.	73.	92.	117.	123.	695
F.	Control...........	0	14.5	32.	49.5	64.	85.5	103.	107.	638
F.	Hypophysis.......	0.05	13.	31.5	50.	66.5	87.	105.	111.	754
F.	Hypophysis.......	0.05	15.	34.5	50.	65.	81.	[illegible]	110.	633
F.	Hypophysis.......	0.05	13.	31.5	49.	58.5	85.	110	108.5	735
F.	Control...........	0	12.	28.	43.5	54.	72.	96.	[illegible]	666

TABLE XII

(LITTER 7)

Sex	Feeding	Tail length	Body length	Testes	Ratio of testes to body weight
M.	Control....................	15.4	16.4	1.78	1.37
F.	Control....................	14.4	16.4	....	....
M.	Hypophysis...............	14.4	15.6	1.91	1.69
F.	Hypophysis...............	15.3	15.5	....	....
M.	Control....................	....	....	1.87	1.52
F.	Control.................	....	....	....	....
F.	Hypophysis...............	14.5	15.4	....	....
F.	Hypophysis...............	...	....	....	....
F.	Hypophysis...............	14.3	14.9	...	....
F.	Control....................	14.5	15.2	....	....

NOTES.—These animals were observed frequently during the experiment and no differences in their development, such as sexual precocity or premature descent of the testes was noted. At the end of the experiment all the animals were active and showed no evidence of snuffles.

Microscopical Findings.—Testes (hypophysis-fed). All sections show evidence of active spermatogenesis with numerous spermatids and almost fully developed spermatozoa.

Epididymis. The tubules are filled with cellular detritus and a few spermatozoa. Ovaries: Sections show Graafian follicles in various stages but no corpora lutea. Uteri (cornua): The mucosa is moderately hyperplastic and there is active glandular proliferation.

Control: Sections taken from areas corresponding to those of the hypophysis-fed rats show almost identical findings.

TABLE XIII

(LITTER 8)

Born Feb. 24, 1917. (Seven animals.)
Feedings begun Mar. 17, 1917. 21 days old.
Animals killed Apr. 28, 1917. 63 " "
Duration of experiment, 40 days.

Sex	Feeding	Dose	Wght. Mar. 16	Wght. Mar. 21	Wght. Mar. 31	Dose Mar. 31	Wght. Apr. 7	Wght. Apr. 12	Wght. Apr. 21	Wght. Apr. 28	Percentage Wght. gained
M.	Hypophysis...	0.05	15.7	25.7	31.7	0.1	36.4	43.2	51.7	66.4	322
F.	Hypophysis...	0.05	15.7	27.7	34.3	0.1	38 1	44.	52.1	66.1	321
M.	Hypophysis...	0.05	16.8	27.7	32.5	0.1	37.5	44.5	53.7	65.3	285
F.	Control.......	0	16.3	27.1	33.2	0	37.3	43.1	51.2	62.8	285
M.	Control.......	0	16.1	27.2	31.1	0	37.5	42.4	50.	61.8	286
M.	Thymus.......	0.05	15.6	28.6	34.3	0.1	42.	50.	63.5	78.1	400
M.	Thymus.......	0.05	15.6	27.5	33.1	0.1	41.6	47.4	56.2	69.8	341

TABLE XIV

(LITTER 8)

Sex	Feeding	Tail Length	Body Length	April 12 Weight of removed testes	April 28 Testes	Ratio of testes to body weight
M.	Hypophysis.....	11.4	13.4	0.357	0.732	....
F.	Hypophysis.....	11.5	13.8	....	....	....
M.	Hypophysis.....	11.3	14.1	....	1.425	2.18
F.	Control.........	11.6	13.5	....	....	....
M.	Control.........	11.6	14.2	0.385	0.755	....
F.	Thymus.........	13.	14.3	....	1.67	2.13
M.	Thymus.........	11.2	14.2	0.421	0.842	....

NOTES.—*March 31.* All the animals are active; no evidence of snuffles. The testes of the thymus-fed rats are fully descended, while those of the hypophysis-fed and control rats are but partially descended. No variation in fur or size of body.

April 6. Animals in excellent condition. Testes of thymus-fed are somewhat larger than others. Mammæ of all females are equally developed.

April 12. Right testes of each male removed for histological study.

April 19. Animals in excellent condition, fur sleek and thick. No appreciable difference except for general increase in size of thymus-fed animals.

Microscopical: Testes (hypophysis-fed rats six weeks old) removed April 12. The tubules are filled with spermatogonia and spermatocytes. An occasional spermatid is seen. Section from rat receiving no gland substance shows the same finding. Sections from the testes of the thymus-fed rats show spermatogonia, spermatocytes and many spermatids.

Sections of testes of hypophysis-fed and control rats taken at autopsy all show active spermatogenesis and fully developed organs. Similarly the female sexual organs of all animals show fully developed organs.

TABLE XV

(LITTER 9)

Born Feb. 27, 1917. (Seven animals.)
Feedings begun Mar. 17, 1917. 18 days old.
Animals killed May 6, 1917. 68 " "
Duration of experiment, 50 days.

Sex	Feeding	Dose	Wght. Mar. 17	Wght. Mar. 24	Wght. Mar. 31	Dose Mar. 31	Wght. Apr. 7	Wght. Apr. 15	Wght. Apr. 21	Wght. Apr. 28	Wght. May 5	Percentage wght. gained
F.	Hypophysis...	0.05	14.	22.3	26.	0.1	33.	41.	46.	59.	76.5	443
M.	Hypophysis...	0 05	14.3	25.6	31.6	0.1	36.9	46.5	52.7	68.7	84.3	499
M.	Hypophysis ..	0.05	15.6	25.1	31.3	0.1	39.8	50.	58.6	72.2	87.7	462
M.	Thymus.......	0.05	14.1	26.1	31.8	0.1	39.8	48.2	52.	68.	78.7	458
F.	Thymus.......	0.05	13.8	25.8	30.8	0.1	39.8	50.	57.5	71.6	82.5	498
M.	Control.......	0	14.2	21.4	25.8	0	29.	37.2	43.3	52.8	63.5	347
F.	Control.......	0	13.8	26.	31.2	0	36.6	44.5	51.2	61.4	74.	436

TABLE XVI

(LITTER 9)

Sex	Feeding	Tail length	Body length	April 20, removed testes	May 6, testes
F.	Hypophysis.................	12.4	14.5	....	....
M.	Hypophysis.................	12.8	15.4	0.54	0.933
M.	Hypophysis...	12.9	15.8	....	1.555
M.	Thymus..........	12.2	14.5	0.51	0.87
F.	Thymus..........	12.7	14.6	....	....
M.	Control..........	12.	14.1	0.33	0.735
F.	Control.....................	12.1	15.	...	....

NOTES: *March 28.* Animals in excellent condition. The testes of the hypophysis-fed animals partially descended, of thymus-fed fully descended. Testes of control not felt.

April 7. Animals in excellent condition; no differences in activities. Fur of fed animals and control animals alike. The testes of the thymus-fed animals are slightly larger than those of the control animals.

April 12. Testes of each pair removed for microscopical study.

April 28. The controls are smaller than the gland-fed animals. All are well nourished and active. No differences can be made out in fur or genitalia.

May 5. Rats seem well; no evidence of snuffles made out. Feedings as last noted.

Microscopical: Testes from hypophysis-fed rats removed at six weeks of age show tubules completely filled with spermatogonia, spermatocytes, and an occasional spermatid generally situated in the outer zone of spermatocytes.

The testes of the rats fed no gland show similar findings. Sections of testes of hypophysis-fed and control rats removed at autopsy show fully developed organs. Evidences of spermatogenesis are equally active in both. Ovaries, Fallopian tubes, uteri of the hypophysis-fed animals show fully developed organs. The control animals show similar findings.

FIG. 4.—Photograph of (1) hypophysis-fed male rat, weight 69 gms., (2) control male rat, weight 66.5 gms., of litter 2.

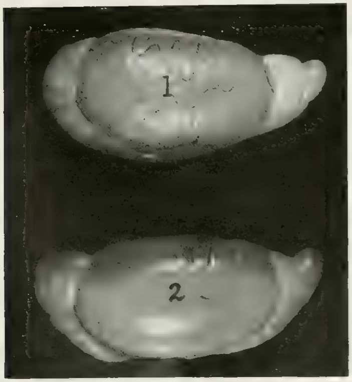
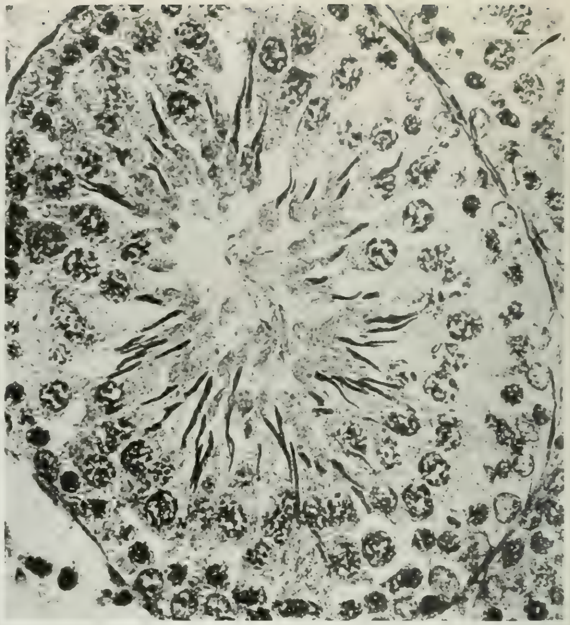
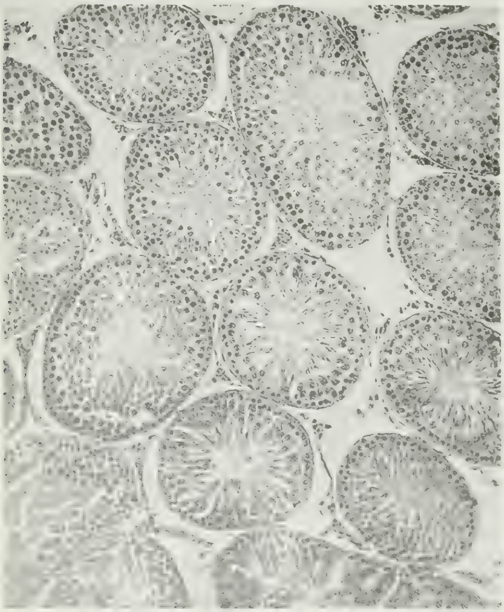


FIG. 5.—Photograph of testes of (1) control rat, (2) hypophysis-fed rat, of litter 1. Weight of testis of control rat, 2.26 gms., of hypophysis-fed rat 2.32 gms.

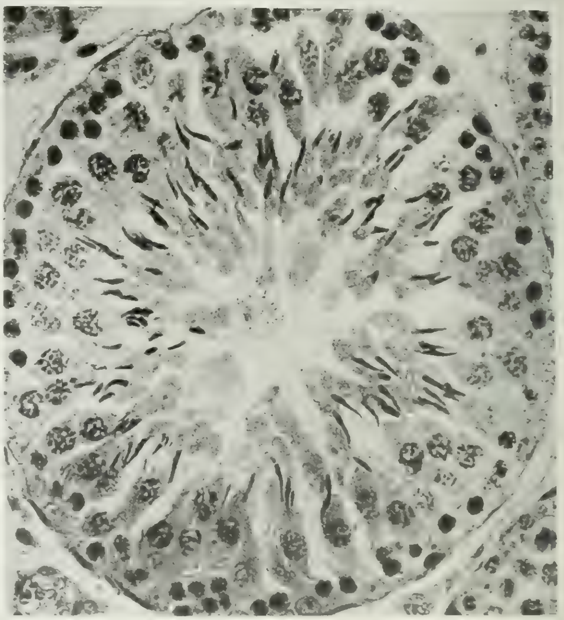
1



1A



2



2 A

Fig. 6.—Microphotographs of testes of (1) and (1A) hypophysis-fed rat, (2) and (2A) control rat of litter 9, showing no differences in spermatogenesis after 35 days of feeding.

TABLE XVII

(LITTER 10)

Born Feb. 23, 1917. (Seven animals.)
Feedings begun Mar. 16, 1917. 21 days old.
Animals killed May 12, 1917. 78 " "
Duration of experiment, 57 days.

Sex	Feeding	Dosage Mar. 16	Wght. Mar. 16	Wght. Mar. 24	Wght. Mar. 31	Wght. Apr. 5	Dosage Apr. 5	Wght. Apr. 11	Dosage Apr. 11	Wght. Apr. 19	Wght. Apr. 25	Wght. May 1	Dosage May 1	Wght. May 12	Percentage gained
M.	Control.....	0	24.	22.	32.	43.4	0	52.5	0	61.	72.1	87.	0	106.	341.7
F.	Control.....	0	20.	18.5	22.8	29.6	0	35.6	0	46.6	54.6	66.5	0	83.6	318.
M.	Hypophysis.	0.05	23.	22.	33.	38.7	0.1	45.1	0 2	49.	66.8	80.8	0.3	107.1	365.6
F.	Hypophysis.	0.05	19.	16.5	27.5	34.6	0.1	43.3	0.2	50.5	64.8	75.2	0.3	89.2	368.5
M.	Thymus.....	0.05	23.	21.7	31.	38.4	0.1	42.1	0.2	57.	69.6	83.7	0.3	106.	360.8
F.	Thymus.....	0.05	22.	20.5	34.	40.	0.1	44.9	0.2	Killed, by accident, Apr. 15				....	....
F.	Thymus.....	0.05	23.	21.	28.5	34.2	0.1	38.1	0.2	45.6	58.4	72.2	0.3	91.	295.5

NOTES: *March 29.* Animals have done very poorly; not especially vigorous. All have a diarrhœa. Testes not descended.

April 11. Marked improvement in condition of all animals, no diarrhœa. Testes descended; no differences in genitals noted.

April 25. Animals observed daily since last note; no differences in activity, fur or size noted. No snuffles.

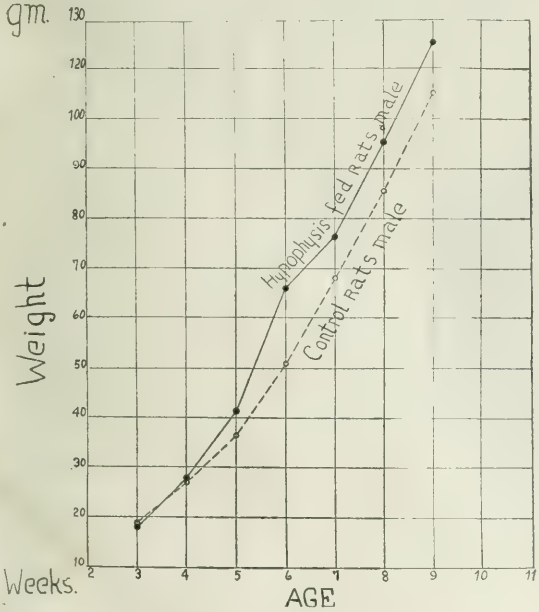


FIG. 1.—Showing combined weight curves of hypophysis-fed and control male rats of litters 1–6, on diet of mixed grains and bread and milk, fed in unlimited amounts.

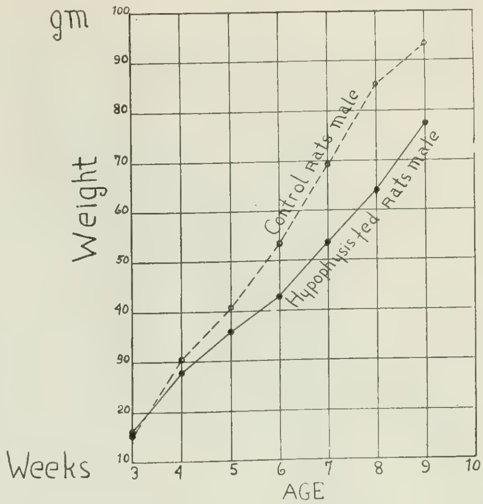


FIG. 2.—Showing combined weight curves of hypophysis-fed and control male rats of litters 7–9, on unlimited amounts of mixed grains and equal amounts of milk.

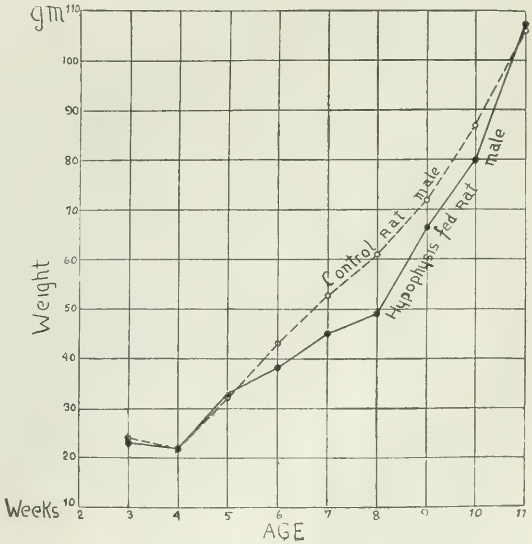


FIG. 3.—Showing weight curve of hypophysis-fed and control male rat of litter 10, on balanced diet of purified casein, dextrin, salts and food accessories, given in equal amounts to each rat.

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SYPHILITIC RUPTURE OF A PAPILLARY MUSCLE OF THE HEART

By Edward D. Spalding and William C. Von Glahn

(*From the Pathological Department, City Hospitals, Baltimore, Md.*)

The following is a case report of one of the rarer cardiac accidents.

S. H. (6650M), colored, male, laborer, age 31, admitted and died March 23, 1920.

F. H. and P. H. Unessential except for a small venereal sore 1905, and one miscarriage by wife.

P. I.—Following a three weeks' attack of hoarseness 15 months before admission, the patient began to be noticeably dyspnœic and orthopnœic, and developed a nocturnal cough with blood-streaked sputum. This subsided spontaneously, but returned more severely a year later, when he was admitted to the University of Maryland Hospital, January 5, 1920, at which time the following data were recorded:

P. E.—The heart was not enlarged to the right, but the apex was in the sixth interspace, 14 cm. to the left, where an indefinite presystolic thrill was felt. There was a diffuse precordial heave. At the apex, the cardiac sounds were replaced by a well transmitted, blowing systolic and short diastolic murmur. At the base and down the sternum a short diastolic was heard, replacing the aortic second sound. The second pulmonic was accentuated. The pulse was regular, Corrigan in type. B. P. 130/50.

Slight dullness was present at the extreme base of the right lung behind, and the liver was three fingerbreadths below the C. M. but not tender. There was some œdema of the ankles.

Laboratory Findings.—Urine, S. G. 1.011–33, negative for albumin and casts. Phthalein excretion, 24 per cent first hour. Blood.—Slight secondary anæmia; Wassermann, + + + +.

X-Ray Findings.—Enlarged heart and aorta; clouding at the base of right lung.

Course.—Gradual restoration of compensation, with two minor remissions. Discharged February 27, 1920.

Subsequent Course.—After discharge the patient gradually went down-hill again, and after one month was admitted to the City Hospital for treatment.

On admission he was moderately œdematous, and dyspnœic. His pulse was rather rapid, and collapsing in character but regular and of fair quality. He did not appear to be in a grave condition and was given a tub-bath on the ward. Seen immediately afterward by the ward physician, he complained of feeling badly. He was cyanotic, sweating, and very dyspnœic, with a very rapid, thin, weak pulse. He was wheeled to his bed, and three minutes later was frothing at the nose and mouth. His heart was found to be greatly dilated, chiefly to the left. Venesection was immediately done, strophanthin administered intravenously, and morphin and atropin given subcutaneously. The pulse became somewhat stronger, the respirations were slow and very labored with tracheal rhonchi. The patient was turned on his face and artificial respiration resorted to, but in spite of these measures he very shortly died. The blood Wassermann was subsequently reported as + + + +.

Clinical Impression.—1. Syphilis. 2. Aortic insufficiency (syphilitic). 3. Acute myocardial insufficiency with pulmonary œdema.

Autopsy No. 1625. *Anatomical Diagnosis.*—Syphilis; syphilitic aortitis with involvement of aortic valve; aortic insufficiency; fibrous myocarditis; cardiac hypertrophy and dilatation; chronic passive congestion of viscera; œdema of legs; necrosis of posterior mitral papillary muscle with rupture; pulmonary œdema.

Heart.—The pericardial cavity does not contain an excess of fluid and its surfaces are smooth and glistening. On the anterior surface of the ventricles are several tendinous patches. The heart weighs 560 grams. The right auricle is slightly dilated. The foramen ovale is closed. The right ventricle is hypertrophied and dilated. It measures 10 cm. from the tricuspid ring to the apex. The tricuspid and pulmonary valves are normal and their rings measure respectively 12 and 6.5 cm. in circumference. The wall of the ventricle is 1 cm. thick.

The left auricle is hypertrophied and dilated. The mitral valve is thin and delicate and its ring measures 9 cm. in circumference. The cavity of the ventricle is much enlarged, measuring 12 cm. from the mitral ring to the apex. The columnæ carneæ stand out prominently. The wall of the ventricle is 2 cm. in thickness. The posterior (or inferior) papillary muscle is Y-shaped, being divided into two large pillars. That pillar to which the chordæ tendineæ from the right half of the aortic leaflet of the mitral valve are attached, has ruptured close to the point of the division (Fig. 1.) The fragment attached to the valve is yellowish white in color and the endocardium over it is smooth but opaque. The torn end is rough, irregularly conical in shape, and is covered with a firmly adherent clot. In the central portion there is still recognizable a core of muscle encircled by a yellow opaque ring, which lies just beneath the endocardium. The stump is likewise covered with smooth endocardium and is opaque and quite yellow in color. The line of demarcation is very sharp and is emphasized by a narrow zone of hemorrhage. The central part of this fragment is softened. The other pillar of this papillary muscle and the anterior papillary muscle are covered with opaque endocardium, beneath which are a few petechiae. On section there are numerous yellow flecks and silvery grey streaks in these muscles. The endocardium is thickened below the aortic valve and also over some of the columnæ carneæ, where there are many small subendocardial hemorrhages. The myocardium is greyish red in color and the muscle fibres are hypertrophied. In the wall of the aorta just above the attachment of the left posterior and the anterior leaflets of the aortic valve, is a large, oval, corrugated plaque, which extends downward, involving these two cusps. The anterior half of the left posterior leaflet has been entirely destroyed, though the line where it was attached is still visible as a little ridge. The remnant of this cusp is moderately thickened. The left half of the anterior leaflet is also eroded but to a less extent. The margin of this cusp is thickened and rolled over. The aortic ring measures 7 cm. in circumference.

The orifices of the coronary arteries are not encroached upon by the plaque in the wall of the aorta, and these arteries appear normal as far as they can be followed.

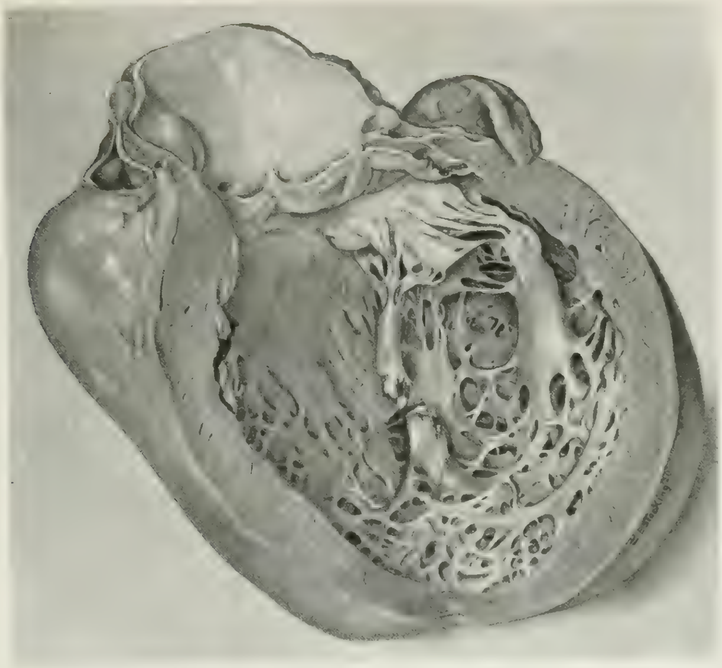

Fig. 1.—Rupture of Papillary Muscle. Note also the erosion of the aortic valve.

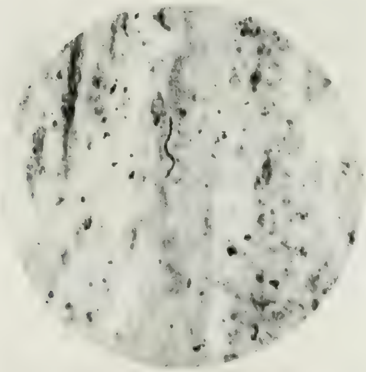

Fig. 2. Levaditi preparation. Spirochæte at margin of the necrotic tissue. × 820.

Microscopic Examination.—The section is from the stump of the ruptured muscle. The central part is made up of a large area of coagulative necrosis in which is much nuclear dust, and about the margin of this necrotic area leucocytes have accumulated. Just beyond the leucocytes, the muscle fibres are shrunken almost to threads and the striations and nuclei have disappeared in them. Small hemorrhages are found among these atrophied fibres. Next to these atrophic fibres is a zone of muscle fibres containing fat droplets. In many of these fibres the nuclei are pyknotic; in others, the nuclei are large and hyperchromatic with irregular outlines. The blood vessels in this zone are greatly engorged. Here the process ends abruptly.

Beneath the endocardium is a small amount of hyaline material into which a few fibroblasts are growing. A very few plasma cells and small round cells are seen here.

The muscle fibres elsewhere are greatly hypertrophied and there is an increase in connective tissue.

In Levaditi preparations a moderate number of spirochætes are found in the tissues just beyond the necrotic area (Fig. 2).

The histological changes seen in the ruptured muscle differ in many respects from the classical picture of a gumma. In the area of coagulative necrosis, all outlines of the preexisting tissue are completely obliterated. Neither are there any epithelioid or giant cells. Accumulations of small mononuclear wandering cells are entirely wanting and the connective-tissue capsule so commonly seen about gummata is also absent.

In a section of the anterior papillary muscle, there is atrophy with hyalinization of some of the muscle fibres and hypertrophy of others. Small hemorrhages are found among the atrophic fibres. The connective tissue is increased. There is no plasma or small-round-cell infiltration.

Literature—Wankel, in 1911, reported a case of ruptured papillary muscle, and in a review of the literature found only four other cases. Teacher the same year also reported a case. In all of these, the ruptured muscle was in the left ventricle.

In four of the cases—those of Bertin, Dennig, Wankel and Teacher—there was sclerosis of the coronary arteries, and in two of these (Dennig, Wankel) there was thrombosis of these vessels. In the case of Corvisart, no mention is made of the condition of the coronary arteries, while in Legendre's case they were normal. In none is there any reference to a syphilitic infection, but an aneurysm of the aorta was present in Bertin's case.

Conclusion—In the case reported here, the Treponema pallidum was the etiological agent in the production of the necrosis of the papillary muscle which subsequently ruptured.

We are indebted to The University of Maryland Hospital for the abstract of their history, and to Dr. E. H. Terrill for the photograph.

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NOTES AND NEWS

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NOTES ON NEW BOOKS

The Diagnosis of Nervous Diseases. By SIR JAMES PURVES STEWART, M. D. Fifth Edition. Cloth, $11.00. (*New York: E. B. Treat & Co., 1920.*)

This edition follows the arrangement of the previous one published four years ago. The chapter titles are based on anatomic and physiologic divisions of the nervous system (with the two exceptions of Aphasia and Brain Tumor) instead of on disease groups, thus permitting reference to various chapters—for instance, those dealing with all disturbances of incoordination, changes in cerebrospinal fluid, or organic motor paralysis of the lower neurone type.

This grouping of signs and symptoms corresponds with the correct thought processes in diagnosis. It is convenient for the neurologist, and should be a stimulus to the student to study a case-problem free from the pigeonholing constraint of disease groups.

The chapters on reflexes are well illustrated with methods of eliciting them, and the exposition of the electro-diagnosis and electro-prognosis is unusually full and clear (13 pages). The use of the condenser, popularized during the war, is explained; the fact of formula reversal in RD is certainly disputable, and its value as a phase of RD would be denied by some.

The illustrations are excellent, nearly 300 figures, and the charts are good, especially those in the sections on Anatomy and Physiology (Chapters 1 and 2).

The index is adequate. The book belongs among the few first-class ones on its subject.

Diagnosis and Treatment of Brain Injuries. By WILLIAM SHARPE. (*Philadelphia and London: Lippincott Company, 1920.*)

The monograph deals entirely with the intracranial complications of skull injuries. The author first describes his operative methods (without giving credit to his preceptors) and then reports case after case of acute and chronic brain injury. The case reports are long, and many of them are not of enough interest to hold the reader's attention. The last section deals with birth trauma and is of considerable importance, for the author has collected a large number of cases which he has treated with varying success. V. R. M.

Studies in Neurology. In two volumes. By HENRY HEAD. Cloth, $17.00. (*London: Henry Frowde, 1920.*)

The two volumes contain the studies on sensation by Henry Head and his associates. They represent the result of many years of wearisome and painstaking clinical research. No review of the subject matter is necessary, for the studies are well known to all who are interested in the advancement of medical diagnosis. The monograph is a milestone in the progress of neurology and a credit to its author. V. R. M.

PUBLICATIONS

The following twelve monographs:

Benzol as a Leucotoxin. By LAURENCE SELLING, M. D. 60 pages. Price, $1.00.

Primary Carcinoma of the Liver. By M. C. WINTERNITZ, M. D. 42 pages. Price 75 cents.

The Statistical Experience Data of The Johns Hopkins Hospital, Baltimore, Md., 1892-1911. By FREDERICK L. HOFFMAN, LL.D., F.S.S. 161 pages. Price, $2.00.

Venous Thrombosis During Myocardial Insufficiency. By FRANK J. SLADEN, M. D., and MILTON C. WINTERNITZ, M. D. Price, 75 cents.

The Origin and Development of the Lymphatic System. By FLORENCE R. SABIN. 94 pages. Price, $2.00.

Leukaemia of the Fowl: Spontaneous and Experimental. By HARRY C. SCHMEISSER, M. D. Price, $2.00.

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The Operative Story of Goitre. The Author's Operation. By WILLIAM S. HALSTED, M. D. Price, $3.50.

Study of Arterio-Venous Fistula with an Analysis of 447 Cases. By CURLE L. CALLANDER, M. D. Price, $2.50.

Ligations of the Left Subclavian Artery in its First Portion. By WILLIAM S. HALSTED. Price, $2.00.

The Pathology of the Pneumonia in the United States Army Camps During the Winter of 1917-18. By WILLIAM G. MACCALLUM. Price, $1.50.

Pathological Anatomy of Pneumonia Associated with Influenza. By WILLIAM G. MACCALLUM. Price, $1.50. (This monograph will be on sale within a short time.)

are now on sale by THE JOHN'S HOPKINS PRESS, Baltimore. Other monographs will appear from time to time.

BULLETIN

OF

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(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

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CONTENTS

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THE SIGNIFICANCE OF THE BACTERIA FOUND IN THE THROATS OF HEALTHY PEOPLE

By ARTHUR L. BLOOMFIELD

(*From the Biological Division of the Medical Clinic, The Johns Hopkins University and Hospital*)

In studying the etiology and pathogenesis of infectious diseases the problem is simplified vastly by the existence of a lesion from which the virus may be recovered in pure culture. Meningitis and sepsis, conditions which previously were but incompletely understood, immediately became intelligible from the bacteriological standpoint following the introduction of spinal puncture and blood cultures which made it possible to study the organisms derived in pure culture from the seat of the disease. The causal agents of respiratory disease, on the other hand, have proven much more elusive, and the proof of their relation to particular lesions is much more difficult, since the ground is already occupied by organisms not primarily causing the process, or at least is open to secondary invasion from the upper air passages by a host of unessential organisms. It required several years to establish the pneumococcus as the cause of lobar pneumonia, and a generation later we find the problem of the etiology of influenza even more perplexing and thus far insoluble. In reviewing the history of the latter disease it becomes apparent that confusion has arisen through the focussing of the investigator's attention upon one or another organism without due consideration of the flora as a whole both in health and in disease.

During the course of studies on the fate of bacteria introduced into the upper air passages it became apparent that foreign organisms were rapidly eliminated. It seemed probable, therefore, that the true normal flora of these regions might be relatively simple and constant, and that, if it could be clearly defined, a background would be obtained against which the flora present in various disease conditions might reasonably be contrasted with less chance of misinterpretation. For example, should it turn out that the green streptococcus is constantly present in normal throats, its presence in disease would have to be interpreted with great caution, whereas an organism not normally present in healthy throats, if persistently found in a given individual, would indicate disease or at least an abnormal carrier state. It immediately became clear that a large number of single cultures from various individuals would help but little in the solution of this problem. The literature already affords ample information as to the great variety of organisms which may be recovered from the apparently normal throat. Thus, it appeared more hopeful to study the flora of a few individuals repeatedly at frequent intervals over a considerable period of time, as by this means one could determine not only the organisms present, but their persistence as well as their relative predominance. Moreover, chance temporary invaders could be distinguished from bacteria actually dwelling in the mouth cavity.

Methods

Six healthy individuals were selected for study. They included the physicians and personnel working in the bacteriological laboratory, and being constantly and intimately exposed to infectious diseases of all sorts. In addition, a few patients not suffering from disease of the respiratory tract were investigated. Cultures were made about once a week from each of these individuals over a period of from one to three months. A swab was systematically passed over the tonsils, pillars, soft palate, and posterior pharyngeal wall and immediately plated. Aërobic methods only were used. The media consisted of plates of 5 per cent blood agar (human or rabbit) and of Avery's oleate hemoglobin medium. Four plates were made from each swab, a system of dilutions being employed which yielded a good spread of colonies on the plates. Cultures as ordinarily made, resulting in a confluent growth, are useless in this sort of work. The plates were studied after 24 and after 48 hours from two points of view. First a careful search was made for the presence of *B influenzæ, Strept. hemolyticus,* the meningococcus, pneumococcus and staphylococcus albus and aureus. Then a study was made of the other organisms present.. Every type of colony present was fished and the numbers of each estimated as accurately as possible. The cultures were worked out in great detail, about four hours being found necessary for the complete study of one set of plates.

Results

It soon became apparent that certain organisms were almost constantly present from day to day. Before analyzing the results in detail a few general remarks may be made about each of the groups of organisms present.

(1) *The Gram-Negative Cocci.*—In practically every plate the predominating organisms were found to belong to the group of Gram-negative cocci. At first an attempt was made to differentiate several varieties and strains on the basis of colony size and consistency, pigmentation, and growth on various media. This task was soon abandoned as impossible, inasmuch as every variation was found, from small translucent grey colonies to large, dry crinkly pigmented ones. Finally, the study of this group was confined to the elimination of the meningococcus, the remaining organisms being classed simply as Gram-negative cocci. As one becomes familiar with the appearance of plates made by a constant method, the colonies of these bacteria stand out as a characteristic background. With the exception of a few cases which will be discussed in connection with the protocols they were present in every culture and undoubtedly are a constant and normal inhabitant of the upper air passages.

(2) *The Non-Hemolytic Streptococci.*—These organisms as well as the Gram-negative cocci were present in nearly every culture. Undoubtedly many strains exist—as evidenced by variation in appearance of the colonies, and in the morphology of the organisms. The usual type is a small grey semi-transparent colony 0.5 to 1 mm. in diameter with a slight greenish zone of clearing; microscopically, the bacteria are usually in long chains and show considerable pleomorphism. Organisms belonging to this group are undoubtedly constant inhabitants of the normal throat, although temporarily they may be replaced by other bacteria.

(3) *Influenza Bacilli.*—Members of the group of hemophilic Gram-negative bacilli were found in most of the cultures. The position of this organism is still in doubt, and inasmuch as these studies were made at a time when respiratory disease was prevalent one hesitates to draw final conclusions. A special study of this problem is now in progress. It may be said, however, that Gram-negative hemophiles may be present in large numbers in the throats of healthy people constantly and over considerable periods of time.

The above three groups seem to comprise very definitely the constant normal flora of the mouth when cultures are made by this method. We are inclined to believe that the presence of any other organism demands a special explanation—either the presence of disease, a focus of latent infection, or accidental introduction. Before presenting the protocols a few general remarks may be made about the other organisms considered in this study.

Hemolytic streptococci of the typical beta type were not obtained in any case. This is of interest, as the cultures were made from people who were in intimate contact with patients suffering from sore throats due to these organisms. We believe that their presence in normal throats, as reported by some writers, indicates the recent presence of widespread infection among large groups of people with a virulent type of the germ. Hemolytic streptococci of the alpha type were occasionally found but never as constant inhabitants. Their significance will be discussed in connection with the individual cases.

Pneumococci were occasionally found. Inasmuch as mouse inoculation was not employed, their presence may have been overlooked in some cases. All suspicious colonies were carefully studied, however, and this organism is certainly not a member of the normal flora in the strict sense.

Meningococcus was found in no instance. This was to be expected as there was no meningitis in the environment at the time.

Staphylococci were found occasionally, but never constantly, in the same normal throat. This organism, therefore, when present, is an accidentally introduced transient unless associated with a local infection.

Diphtheroid bacilli, Gram-positive cocci of various sorts, and other organisms, as described in the protocols, are not uncommonly present, but only as transients.

The non-pathogens in the throat may, therefore, be divided into the members of the constant normal flora—Gram-negative cocci, non-hemolytic streptococci, Gram-negative hemophiles (?)—and a group of organisms commonly present about the body such as staphylococci, diphtheroids, and others, which not infrequently may be introduced into the mouth but are transients and are promptly eliminated.

Protocols

A scrutiny of the protocols herewith presented will bring out more clearly the points discussed above.

CASE I.—MO

Date of culture	Non-hemolytic streptococcus	Gram-negative cocci	Hemolytic streptococcus	Pneumococcus	Influenza bacillus	Staph. albus	Meningococcus	Remarks
Mar. 13..	∞*	A few.	0	0	0	0	0	A Gram-pos. diphtheroid, 20 cols.
" 23..	100	Many.	0	Type IV, a few cols.	A few.	0	0	
" 31..	Many.	Many.	0	Type IV, a few cols.	∞	0	0	
Apr. 6..	∞	∞	0	0	Many.	0	0	
" 21..	Many.	∞	0	0	0	∞	0	
" 26..	∞	∞	0	0	Many.	0	0	

Comment.—The non-hemolytic streptococci and Gram-negative cocci were constantly present. Influenza bacilli were recovered in four of six cultures. A Type IV pneumococcus was temporarily present, and on one occasion Staph. albus was recovered. A diphtheroid was present in the first culture, but was never isolated again.

In this case, therefore, we have the constant normal flora, with the occasional transient presence of other non-pathogenic organisms.

* ∞ = innumerable.

CASE II.—CA

Date of culture	Non-hemolytic streptococcus	Gram-negative cocci	Hemolytic streptococcus	Pneumococcus	Influenza bacillus	Staph. albus	Meningococcus	Remarks
Feb. 24..	200	∞	0	0	200	0	0	A large Gram-pos. coccus, 200 cols.
" 27..	Few.	∞	0	0	300	0	0	A large Gram-pos. coccus, a few cols.
Mar. 3..	10	∞	0	0	A few.	0	0	A yeast, 1 col.
" 10..	∞	Many.	0	0	A few.	0	0	
" 17..	∞	∞	Many cols. alpha type	0	A few.	0	0	
" 24..	∞	∞	0	0	100	0	0	

Comment.—The non-hemolytic streptococci, the Gram-negative cocci and influenza bacilli are constantly present. An alpha type hemolytic streptococcus was recovered in one culture, but not before or after. A large Gram-positive coccus was present for a few days and then disappeared. We have, therefore, in this case the normal flora plus the transient presence of other organisms.

CASE III.—BL

Date of culture	Non-hemolytic streptococcus	Gran-negative cocci	Hemolytic streptococcus	Pneumococcus	Influenza bacillus	Staph. albus	Meningococcus	Remarks
Mar. 4..	∞	∞	Alpha type 100 cols.	0	A few.	0	0	A Gram-pos. diphtheroid, many cols.
" 8..	A few.	∞	0	0	100	0	0	
" 24..	∞	∞	0	0	Many.	0	0	
Apr. 1..	∞	∞	0	0	Many.	0	0	A Gram-pos. diphtheroid, many cols.
" 8..	∞	∞	0	0	Many.	0	0	Many cols. of a Gram-neg. hemolytic bacillus.
" 26..	Many.	Many.	Alpha type a few cols.	0	Many.	0	0	

Comment.—Non-hemolytic streptococci, Gram-negative cocci, and influenza bacilli were constantly present. Alpha hemolytic streptococcus was present as a transient on two occasions, as well as a gram-positive diphtheroid and a Gram-negative hemolytic bacillus. The significance of the hemolytic streptococcus is uncertain. It may have been harbored in the tonsil whence it was discharged from time to time.

CASE IV.—V

Date of culture	Non-hemolytic streptococcus	Gram-negative cocci	Hemolytic streptococcus	Pneumococcus	Influenza bacillus	Staph. albus	Meningococcus	Remarks
Feb. 28..	∞	80	0	0	∞	0	0	
Mar. 1..	∞	∞	0	0	Many.	1	0	
" 3..	∞	Many.	0	0	100	0	0	1 col. hemolytic staph. Many cols. Gram-pos. diphtheroid. 100 cols. coarse Gram-pos. coccus.
" 6..	Many.	∞	0	0	Many.	0	0	Many cols. Gram-pos. diphtheroid.
" 9..	∞	Many.	0	0	50	0	0	∞ cols. Gram-pos. diphtheroid.

Comment.—Non-hemolytic streptococci, Gram-negative cocci, and influenza bacilli were constantly present. From time to time various other organisms were isolated, especially a Gram-positive diphtheroid.

CASE V.—AL

Date of culture	Non-hemolytic streptococcus	Gram-negative cocci	Hemolytic streptococcus	Pneumococcus	Influenza bacillus	Staph. albus	Meningococcus	Remarks
Jan. 3..	∞	∞	0	0	A few.	Many.	0	Gram-pos. diphtheroid many cols.
Feb. 23..	∞	Several hundred.	0	0	Several hundred.	0	0	Gram-pos. diphtheroid many cols.
" 26..	∞	∞	0	0	∞	0	0	
" 28..	∞	∞	0	0	Many.	0	0	

Comment.—Non-hemolytic streptococci, Gram-negative bacilli, and influenza bacilli were constantly present. Staph. albus was isolated on one occasion, and a Gram-positive diphtheroid twice.

CASE VI.—CA

Date of culture	Non-hemolytic streptococcus	Gram-negative cocci	Hemolytic streptococcus	Pheumococcus	Influenza bacillus	Staph. albus	Meningococcus	Remarks
Feb. 24..	200	∞	0	0	200	0	0	Huge Gram-pos. coccus, 200 cols.
" 27..	A few.	∞	0	0	Several hundred.	0	0	A few of the above.
Mar. 3..	10	∞	0	0	A few.	0	0	A yeast, one col.
" 10..	∞	∞	0	0	0	0	0	
" 17..	∞	∞	Alpha type many.	0	A few.	0	0	
" 25..	∞	∞	0	0	Many.	0	0	

CASE VII.—BV

Date of culture	Non-hemolytic streptococcus	Gram-negative cocci	Hemolytic streptococcus	Pneumococcus	Influenza bacillus	Staph. albus	Meningococcus	Remarks
Feb. 22..	Many.	∞	0	0	∞	0	0	Gram-pos. diphtheroid, 20 cols.
" 25..	∞	∞	0	0	∞	0	0	
" 29..	∞	∞	0	0	∞	0	0	A pigmented staph., 20 cols.
Mar. 2..	∞	∞	0	0	Many.	0	0	A large Gram-pos. coccus, 50 cols.

CASE VIII.—MA

Date of culture	Non-hemolytic streptococcus	Gram-negative cocci	Hemolytic streptococcus	Pneumococcus	Influenza bacillus	Staph. albus	Meningococcus	Remarks
Mar. 10..	∞	∞	0	0	A few.	3	0	
" 29..	∞	∞	0	0	Many.	150	0	
April 7..	∞	∞	0	0	A few.	2	0	
" 27..	∞	∞	0	0	Many.	0	0	A Gram-pos. coccus, not S. albus, many cols.
" 30..	Many.	∞	0	0	Many.	0	0	About 50 cols. of a minute Gram-pos. organism (?).

Comment.—The normal flora is present. In addition Staph. albus was recovered in small numbers in three consecutive cultures, after which it disappeared.

CASE IX.—ER

Date of culture	Non-hemolytic streptococcus	Gram-negative cocci	Hemolytic streptococcus	Pneumococcus	Influenza bacillus	Staph. albus	Mening coccus	Remarks
Mar. 4..	∞	∞	0	0	0	0	0	
" 9..	∞	50	0	0	0	0	0	A Gram-pos. diphtheroid, many cols.
" 16..	∞	∞	Alpha type many cols.	Type IV many cols.	0	0	0	A coarse Gram-pos. coccus, many cols.
" 25..	∞	∞	Alpha type many cols.	0	100	0	0	
Apr. 5..	∞	∞	0	0	A few.	0	0	
" 13..	∞	∞	0	0	0	0	0	Sev. hundred colonies of a Gram-neg. bacillus, large grey cols. with hemolysis.
" 16..	50	Many.	0	0	0	0	0	Many cols. of a coarse Gram-pos. coccus, not S. albus.
" 20..	∞	∞	0	0	Many.	3	0	50 cols. of same Gram-neg. bacillus as found in culture of Apr. 13.
" 25..	∞	∞	0	0	Many.	0	0	

Comment.—The normal flora is present. In addition, influenza bacilli and other bacteria were transiently present. A mild pharyngitis lasting two days preceded the appearance of the alpha hemolytic streptococcus recovered on March 16 and March 25.

CASE X.—GI

Date of culture	Non-hemolytic streptococcus	Gram-negative cocci	Hemolytic streptococcus	Pneumococcus	Influenza bacillus	Staph. albus	Meningococcus	Remarks
Mar. 1..	∞	0	0	0	0	0	0	∞ Friedländer.
" 5..	Many.	A few.	0	0	0	0	0	∞ Friedländer. ∞ Gram-pos. diphtheroid. 50 cols. of a coarse Gram-pos. coccus.
" 12..	∞	A few.	Alpha type many.	0	0	0	0	∞ Friedländer.
" 17..	∞	A few.	0	0	0	0	0	∞ Friedländer.

Comment.—This patient was a carrier of the Friedländer bacillus, which tended to supplant the normal flora.

CASE XI.—DO

Date of culture	Non-hemolytic streptococcus	Gram-negative cocci	Hemolytic streptococcus	Pneumococcus	Influenza bacillus	Staph. albus	Meningococcus	Remarks
Mar. 12..	Many.	20	0	0	3	∞	0	
" 15..	0	100	0	Type II many?	0	∞	0	
" 20..	∞	∞	0	0	0	50	0	A yeast, 50 cols.
" 27..	Many.	∞	Alpha type many.	0	Many.	100	0	
Apr. 2..	Few.	Few.	0	0	Few.	∞	0	
" 12..	Many.	∞	0	0	0	50	0	
" 22..	∞	∞	0	0	0	20	0	Many cols. of a Gram-pos. coccus—not Staph. albus.
Nov. 4..	∞	∞	0	0	A few.	0	0	

Comment.—This person was affected with a subacute rhinitis and pharyngitis following an acute cold. In the first culture the normal flora was practically replaced by a white staphylococcus. As this organism decreased in numbers, the normal flora reappeared in the usual proportions.

Discussion

It appears that cultures made repeatedly from the throat of the healthy individual over a considerable length of time reveal two groups of organisms. In the first place there is a group—non-hemolytic streptococci, and Gram-negative cocci—which is constantly present and seems to constitute the true normal flora of the throat in the sense of actually living its complete life history in that environment. In addition to this group many other organisms may be recovered in cultures from normal throats but their presence seems to be only transient. They disappear within a few days, as a rule, just as foreign organisms do when experimentally introduced. It is possible that some of these organisms such as pneumococci and influenza bacilli are really constant inhabitants, but are present in such small numbers that they were missed in some of the cultures, but this seems unlikely. We may picture, therefore, in addition to the true basic flora a constant influx of various bacteria from the skin, the nose, the air, and from external objects.

These, unless they are pathogenic and set up disease, seem to be promptly disposed of by the normal protective mechanism of the upper air passages (chiefly mechanical flushing.) The idea, then, that bacteria in general, when introduced into the upper air passages are likely to colonize and live there indefinitely is erroneous. It is now clear, both from these studies and from the experimental work, that the normal surfaces of the upper air passages afford a very unfavorable environment for foreign organisms, both pathogenic and non-pathogenic, and that special conditions are needed to make possible their prolonged or permanent presence.[2] Such conditions as a rule consist of the production of disease, or at least a focus of diseased tissue in which the organisms may colonize.

With these points clearly in mind it seems that one is in a much better position to study the bacteriology of infections of the upper respiratory tract. Knowing the normal flora, and the possibility of transient invaders, unessential organisms may be readily discounted. A study of the bacteriology of colds is now being taken up from this point of view.

Conclusions

I. The organisms present in the throats of healthy people, as revealed by this method, fall into two groups:

(a) The true normal flora including non-hemolytic streptococci and Gram-negative cocci, and

(b) Pathogenic or non-pathogenic organisms which are accidentally introduced and are present usually only a short time in a given individual.

II. A true picture of the normal flora is obtained only by making repeated cultures from the same individual.

Reference

1. Bloomfield, A. L.: American Review of Tuberculosis, 1920, IV, p. 247.

EXPERIMENTAL INOCULATION OF HUMAN THROATS WITH AVIRULENT DIPHTHERIA BACILLI

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For a proper appreciation of the relation of the healthy diphtheria bacillus carrier to the community it would be helpful to know which persons are actually carriers or at least the approximate proportion of carriers in the population at large. Next it obviously would be of practical importance to ascertain whether any cases of clinical diphtheria develop among the associates of these carriers.

In a previous communication on this subject[1] we have reported that a single examination of a large series of individuals (2507), both children and adults, in the winter and spring of 1911, revealed an incidence of healthy diphtheria bacillus carriers of about 3.55 per cent. It was also found that a second examination of a large series of persons practically doubled the number of carriers discovered, while a third examination still further increased the original number. It should be pointed out that the percentage of carriers probably varies in different localities in the same year or in the same locality in different years. We found that in the same locality it also varied considerably with the time of the year, being somewhat higher during the winter than in the summer months. Making all allowance for such variation, however, there still exists a number of diphtheria carriers so great as to be out of all proportion to the number of clinical instances of the disease.

With regard to the second point, namely, the relation of these carriers to the occurrence of clinical diphtheria in the community, we were quite unable to discover any instances of the disease among the associates of the carriers studied by us. One hundred and sixty additional carriers were studied the following year (1912) and during the three and a half months covered by our observations no cases of diphtheria were found among their associates.[2]

Investigation also showed that of the total number of healthy carriers under observation—over 200 in all—only 11 per cent (1912) to 18 per cent (1911) harbored virulent diphtheria bacilli. Even 11 to 18 per cent of the total, however, constitutes a number of carriers disproportionately large when compared with the reported incidence of the disease. Despite the fact that we were unable to incriminate any of the carriers under observation in the actual spread of diphtheria, it was recognized, nevertheless, that those who harbored virulent organisms constitute a potential menace to the community.

With regard to the healthy carriers of non-virulent diphtheria bacilli, however, the condition is quite different. Here we have no evidence whatever that any danger exists as a result of the carrier state and on this point we have expressed our conviction strongly.

Two questions are raised from time to time which have a distinct bearing upon the validity of our conclusions concerning the harmless nature of the healthy carrier of non-virulent diphtheria bacilli. The first question concerns the reliability of the guinea-pig test for toxin production by the diphtheria bacillus, with the suggestion that an organism which is non-virulent for the guinea-pig may be in reality virulent for man. The second question is based upon the assumption that a non-virulent strain of *B. diphtheriæ* from the throat of a healthy carrier may become virulent on longer residence in the throat

[1] Moss, W. L., Guthrie, C. G., and Gelien, J.: Diphtheria Bacillus Carriers. Trans. XV Internat. Cong. on Hyg. & Dem., 1912, IV, 156.

[2] Guthrie, C. G., Gelien, J., and Moss, W. L.: Diphtheria Bacillus Carriers, Second Communication. Johns Hopkins Hosp. Bull., 1920. XXXI, 388.

of that carrier, or may acquire virulence when introduced into the throat of another individual. It was to put these and certain other questions to experimental test that the present work was undertaken.

In another place [3] were reported the results of animal experiments designed to show whether the previous injection of diphtheria antitoxin had any effect in preventing the lodgment and growth of diphtheria bacilli, either virulent or non-virulent. Among the facts which seemed apparent from this work were the following:

(1) The production of nasal infection or infestation of cats, rabbits and guinea-pigs with *B. diphtheriæ* was quite inconstant even when the organisms were introduced directly into the nose.

(2) The duration of infection was usually quite short but may have been variable in this respect, as some of the animals still harbored diphtheria organisms at the end of the experiments.

(3) The occurrence and duration of infection were independent of the virulence of the strain of organism used for inoculation and (4) were wholly unaffected by the previous administration of antitoxin.

It was found that certain conditions in these experiments with animals were quite different from those which obtain with human beings; notably that in a considerable series of animals (112) none was found spontaneously infected with diphtheria bacilli and that the carrier state was difficult to induce. Feeling, therefore, that the results of this work might not be entirely applicable to man, further experiments were carried out, this time with human beings instead of animals as subjects.

OBJECT

1. To determine whether the introduction of avirulent diphtheria bacilli into the throats of healthy human beings will result in the production of the carrier state and, if produced, how long it may last.

2. To determine whether the lodgment and growth of the bacilli may be prevented by the previous injection of diphtheria antitoxin.

3. To determine whether the organisms introduced are capable of producing (a) clinical diphtheria; (b) any subjective symptoms; (c) any objective signs in the appearance of the throat.

4. To determine whether any cases of clinical diphtheria develop among the associates of artificially produced "healthy carriers" [4] of virulent diphtheria bacilli.

5. To determine whether the organisms introduced into the throats of normal human beings are in any way changed by this procedure, particularly as to (a) morphology; (b) staining characteristics; (c) cultural characteristics; (d) ability to produce toxin (virulence).

PROCEDURE

Five physicians (W. L. M., C. G. G., S. R. M., A. W. S. and W. A. B.) volunteered to be subjects of these experiments. These individuals, designated for the sake of brevity in the following pages as *A, B, C, D* and *E*—were healthy men varying in age from 28 to 36 years. Only one had had clinical diphtheria and only one had previously received antitoxin; *D* had had a severe attack at the age of five, before the introduction of diphtheria antitoxin; *E* had received a prophylactic injection of antitoxin in 1911. Only one of the five has had diphtheria or received antitoxin since the close of these experiments; *B* had a definite attack the following year (1914) as the result of experimental inoculation with virulent organisms obtained from the throat of a healthy carrier; at this time he received three injections of antitoxin.

Preliminary throat cultures on these five persons were made daily over a period of two weeks to ascertain whether any had diphtheria bacilli in their throats at the beginning of the experiments.

At the end of this preliminary fortnight three members of the group (*C, D* and *E*) received a subcutaneous injection of 250 units of diphtheria antitoxin before being inoculated with the non-virulent diphtheria bacilli. The two remaining members of the group (*A* and *B*) received no antitoxin.

The strain of diphtheria bacillus employed in this work (Culture 48) was obtained by us from a healthy carrier during an investigation of public school children in 1912 and at the time of the experiments reported in the present paper (1913) it had been growing on Loeffler's blood serum for 12 months. When first isolated the strain proved to be non-virulent; repeated tests over a period of a year—15 or more, including those made just at the beginning of this work—in each instance confirmed the original result. Morphologically, tinctorially and culturally the strain was typical *B. diphtheriæ* and was indistinguishable from our virulent strains except by the animal tests for the production of toxin.[5]

The inoculations were made from transplants of this strain grown on Loeffler's blood serum for 24 hours and then suspended in normal salt solution. By using the growth from several tubes, this saline suspension was rendered quite thick and almost milky in appearance.

The inoculations were made by soaking a cotton throat swab in the bacterial suspension and then rubbing it over both tonsils and the posterior pharyngeal wall. As none of the five individuals yielded positive cultures on the following day, a second inoculation was made two days after the first. For this purpose a freshly prepared bacterial suspension was used similar in all respects to the other. This time, however, the suspension was placed in an atomizer and with this the throats of the five individuals were thoroughly sprayed.

[3] Gelien, J., Moss, W. L., and Guthrie, C. G.: The Effect of Diphtheria Antitoxin in Preventing the Lodgment and Growth of the Diphtheria Bacillus in the Nasal Passages of Animals, Johns Hopkins Hosp. Bull., 1920, XXXI, 381.

[4] The term "healthy carrier" is used in distinction to "convalescent" or "contact" carrier.

[5] For further details concerning the methods employed in the identification of this and other strains of *B. diphtheriæ* see reference cited above, "Diphtheria Bacillus Carriers, Second Communication."

Daily cultures were made from four members of the group (*A*, *B*, *C* and *E*) from February 4, 1913, to May 28 of the same year, a period of 114 days or a little over 16 weeks, and then at intervals until July 9, a period of six weeks more. From the other member (*D*) daily cultures were made from February 4, when the experiment began, until he left on an expedition to South America on April 28. Beginning the next autumn, occasional cultures were made from each member of the group until January 12, 1914, when daily cultures were begun again and continued for several months.

The results of the cultures taken from February 4 to July 9, 1913, are shown in the accompanying chronological table (Table I). The blanks in the earlier part of the table indicate days on which members of the group were absent from the laboratory. The table has been condensed from the 120th day onward by omitting the days on which no cultures were taken. In this table a *positive* culture is indicated by + ; a *negative* culture by 0 ; A. T. indicates a subcutaneous injection of diphtheria antitoxin; Inoc. indicates inoculation of the throat with avirulent diphtheria bacilli.

TABLE I

Day	Date	A	B	C	D	E
1	Feb. 4	0	0	0	0	0
2	" 5	0	0	0	0	0
3	" 6	0	0	0	0	0
4	" 7	0	0	0	0	0
5	" 8	0	0	0	0	0
6	" 9	..	..	..	..	..
7	" 10	0	0	0	0	0
8	" 11	0	0	0	0	0
9	" 12	0	0	0	0	0
10	" 13	0	0	0	0	..
11	" 14	0	0	0	0	0
12	" 15	0	0	0	..	..
13	" 16	..	0	..	..	..
				A. T.	A. T.	A. T.
14	" 17	0	0	0	0	0
		Inoc.	Inoc.	Inoc.	Inoc.	Inoc.
15	" 18	0	0	0	0	0
16	" 19	0	+	0	+	..
		Inoc.	Inoc.	Inoc.	Inoc.	
17	" 20	0	+	+	+	0
						Inoc.
18	" 21	0	+	+	+	0
19	" 22	+	+	+	+	..
20	" 23	0	+	..	..	+
21	" 24	0	+	+	+	0
22	" 25	+	+	+	+	+
23	" 26	0	+	+	+	+
24	" 27	0	+	+	+	+
25	" 28	+	+	+	+	+
26	Mar. 1	0	+	+	+	+
27	" 2	+	+	..	+	+
28	" 3	+	+	+	+	+
29	" 4	..	..	..	..	..
30	" 5	+	+	+	+	+
31	" 6	+	+	+	+	+
32	" 7	+	+	+	+	+
33	" 8	+	+	+	+	+
34	" 9	+	+	..	+	+
35	" 10	+	+	+	+	+
36	" 11	+	+	+	+	+
37	" 12	+	+	+	+	+
38	" 13	+	+	+	+	+
39	" 14	+	+	+	+	+
40	" 15	+	+	+	+	+
41	" 16	+	+	..	+	+
42	" 17	+	+	+	+	+
43	" 18	+	+	+	+	+
44	" 19	+	+	+	+	+
45	" 20	+	+	..	+	+
46	" 21	0	0	..	+	+
47	" 22	+	0	..	+	+
48	" 23	0	+	..	..	+
49	" 24	0	0	+	+	+
50	" 25	0	+	..	+	+
51	" 26	+	+	..	+	+
52	" 27	0	0	+	+	+
53	" 28	0	0	+	+	+
54	" 29	0	0	+	+	+
55	" 30	+	0	..	..	+
56	" 31	+	+	+	+	+
57	April 1	+	+	+	+	+
58	" 2	+	0	+	+	+
59	" 3	..	+	+	+	+
60	" 4	..	+	+	..	+
61	" 5	..	0	+	..	+
62	April 6	..	..	..	..	..
63	" 7	..	+	+	+	+
64	" 8	..	0	+	+	+
65	" 9	..	0	+	+	+
66	" 10	..	0	+	+	+
67	" 11	..	0	+	+	0
68	" 12	..	0	+	+	+
69	" 13	..	..	..	..	..
70	" 14	..	0	+	..	+
71	" 15	..	0	+	..	+
72	" 16	..	..	..	..	..
73	" 17	+	+	+	..	+
74	" 18	..	0	+	..	+
75	" 19	..	..	..	..	..
76	" 20	..	..	..	..	..
77	" 21	..	0	+	+	+
78	" 22	0	0	..	+	+
79	" 23	0	0	+	+	+
80	" 24	0	0	+	+	+
81	" 25	..	..	..	..	..
82	" 26	..	..	..	..	..
83	" 27	..	..	..	+	..
84	" 28	0	..	..	+	+
85	" 29	..	..	..	..	..
86	" 30	..	0	+	..	..
87	May 1	0	0	..	..	+
88	" 2	..	..	..	..	..
89	" 3	..	..	..	..	..
90	" 4	..	..	..	..	..
91	" 5	..	0	+	..	+
92	" 6	..	+	+	..	..
93	" 7	..	..	..	..	..
94	" 8	+	0	+	..	0
95	" 9	0	0	+	..	+
96	" 10	..	0	+	..	0
97	" 11	..	..	..	..	..
98	" 12	0	0	+	..	0
99	" 13	+	0	+	..	0
100	" 14	..	0	..	..	+
101	" 15	+	0	+	..	+
102	" 16	..	0	..	..	+
103	" 17	..	0	+	..	+
104	" 18	..	0	..	..	0
105	" 19	..	0	..	..	+
106	" 20	0	0	+	..	+
107	" 21	..	..	..	..	..
108	" 22	0	0	+	..	..
109	" 23	+	..	..	..	+
110	" 24	0	..	..	..	+
111	" 25	..	..	..	..	..
112	" 26	..	0	+	..	..
113	" 27	0	0	+	..	+
114	" 28	..	0	+	..	..
115	" 29	..	..	..	..	..
116	" 30	..	..	..	..	..
117	" 31	..	..	..	..	..
118	June 1	..	..	..	..	..
119	" 2	..	0	+	..	+
120	" 3	..	..	..	..	..
134	" 17	0	0	+	..	+
143	" 26	0	0	+	..	+
144	" 27	..	0	+	..	+
148	July 1	0	0	+	..	0
149	" 2	..	..	..	..	..
156	" 9	..	0	0	..	0

From Table I it will be seen that the preliminary cultures taken daily over a period of two weeks (from February 4 to February 17 inclusive) were negative. We therefore felt that the five individuals who had volunteered for these experiments were not already carriers of diphtheria bacilli.

On February 17, three members of the group (*C, D* and *E*) received 250 units of diphtheria antitoxin subcutaneously. Six hours later these three together with the other two members (*A* and *B*), who had received no antitoxin, were inoculated by swabbing the throats with a saline suspension of avirulent diphtheria bacilli, as described above. Cultures taken on the day following this inoculation proved to be negative in each case, but owing to the time necessary for incubation, the result of these cultures was not known until the day after they were taken, that is, the second day after inoculation of the throats. The negative results having led us to suppose that the inoculation had been ineffective, we reinoculated the entire group by spraying a thick suspension of bacilli into the throat with an atomizer as previously described. Cultures made on the second day after the first inoculation and just before the second inoculation were positive in the cases of two members of the group (*B* and *D*) and it seems not unlikely that the first inoculation alone would have sufficed to establish the carrier state in all five members of the group. Following the second inoculation, the cultures of *B, C* and *D* were regularly positive for many days.

In the case of *A*, the first positive culture did not appear until three days after the second inoculation and regularly positive cultures were not established until eleven days after the second inoculation.

In the case of *E*, the first positive culture was obtained three days after the second inoculation. This was succeeded by one negative culture, after which the cultures became regularly positive.

It is seen, therefore, that as the result of experimental inoculation with avirulent diphtheria bacilli the carrier state was established in all five members of the group, in those who had received a previous inoculation with diphtheria antitoxin (*C, D* and *E*) and in those who had not (*A* and *B*).

The results obtained from the beginning of the experiment on February 4, 1913, to the end of the first series of observations on July 9 of the same year may be summarized from Table I.

From Table II it is seen that the apparent duration of the carrier state as determined by these observations was as follows: *A*, 91 days; *B*, 77 days; *C*, 132 days; *D*, 39 days; and *E*, 125 days. For *A* and *B* this probably represents approximately the actual duration of the carrier state, as cultures from their throats were continued for from six (*A*) to nine weeks (*B*) after the last positive result had been obtained and no further positive cultures were found either during this period or on examination made subsequently to the present series. *D* was a carrier from February 19 until April 28, the date on which he left Baltimore. That he probably remained a carrier for a long time thereafter was shown by the result of cultures taken upon his return in the succeeding fall. *A* and *E* continued to yield positive cultures practically to the end of the first series of observations, recorded in Table I. The last positive culture from *C* was obtained on July 1, followed by one negative on July 9; from *E* the last positive culture was obtained on June 27, followed by negatives on July 1 and 9. That both of them were carriers for a very long time was shown by the result of cultures taken at intervals during the fall of 1913 when those from *E* were occasionally and those from *C* regularly positive.

As mentioned before, daily cultures on the entire group were begun again on January 12, 1914, and continued until May 29 of the same year. In this series *D* showed only one

TABLE II

Cultures	A	B	C	D	E
Total	74	99	81	64	92
Preliminary	12	13	12	11	10
After 1st inoculation with *B. diphtheriæ*.	62	86	69	53	82
After 1st inoculation, before 1st positive.	4	1	3	1	3
After 2d inoculation with *B. diphtheriæ*.	60	84	67	51	80
After 2d inoculation, before 1st positive.	2	0	0	0	1
Date of 1st positive	2–22–13	2–19–13	2–20–15	2–19–13	2–23–13
Date of last positive	5–23–13	5–6–13	7–1–13	4–28–13	6–27–13
Date of last culture	7–1–13	7–9–13	7–9–13	4–28–13	7–9–13
Total positive	32	39	66	52	70
Total cultures after and including 1st positive.	58	85	67	52	79
Per cent positive cultures after 1st inoculation.	51.61%	45.34%	95.65%	98.11%	85.36%
Per cent positive cultures after and including 1st positive.	55.17%	45.88%	98.50%	100%	88.60%

positive culture—that taken on January 17—whereas the cultures from *C* and *E* were frequently positive up to the very end of this series of observations. With regard, then, to the duration of the carrier state as judged from the combined observations extending over 15 months—(February 4, 1913, to May 29, 1914)—it may be said that in *A* it was 91 days, in *B* 77 days, in *D* 11 months, while *C* and *E* were still carriers at the end of 15 months.

It is obvious that the previous administration of 250 units of diphtheria antitoxin did not prevent the lodgment and growth of the non-virulent diphtheria bacilli in the throats of *C, D* and *E*. Indeed, the actual number of positive cultures obtained from them in the first series of observations was much greater (*C*, 66, *D*, 52, *E*, 70) than from *A* and *B*, who had received no antitoxin (*A*, 32, *B*, 39). The same point is emphasized when we compare the percentage of positive cultures (*C*, 95.65%, *D*, 98.11%, *E*, 85.36%; *A*, 51.61%, *B*, 45.34%). The duration of the carrier state also was markedly less in *A* and *B* who did not have antitoxin (*A*, 91 days, *B*, 77 days) than in *C, D* and *E* who received it (*D*, 11 months, *C* and *E*,

15 months plus). We do not wish to imply, however, that the previous administration of antitoxin renders an individual more liable to become a carrier when inoculated with avirulent diphtheria bacilli or that it is responsible for a prolongation of the carrier state when thus induced, but merely to emphasize the fact that in our experiments antitoxin did not prevent the development of the carrier state nor shorten its duration.

Throughout our observations an effort was made to indicate in a general way the relative numbers of the diphtheria organisms seen in the stained preparations from the positive cultures. This was carried out in our records of all but 12 of the 259 positive cultures encountered up to July 9, 1913, by the use of a rough, comparative scale, as follows: (1) Only one or two diphtheria bacilli seen. (2) Very scanty. (3) Scanty. (4) Moderate number. (5) Numerous. (6) Very numerous. (7) Almost pure culture. These comparative figures are omitted in Table I since they distract attention from the much more important point as to whether a given culture is positive or negative and might be interpreted as implying much more than we feel they deserve or intend to suggest. A study of these records, however, revealed certain points of interest, confirming general impressions which we had gained from previous studies of carriers. When the carrier state was once firmly established and the cultures were regularly positive each day, the organisms were recovered in great abundance. Before the carrier state was firmly established, as evidenced by occasional negative cultures, the organisms were recovered in fewer numbers. After what might be called the height of the carrier state and when occasional negative cultures were obtained, there were great fluctuations in the number of organisms recovered. Finally, it appeared that before the organisms disappeared entirely from the throat they became scanty, were consequently much less apt to be recovered and considerable intervals elapsed between the positive cultures.

When we come to consider the effect of the inoculation with avirulent organisms on the health of these five men, we are at once confronted by the question whether any of them possessed natural immunity against diphtheria toxin and so may have escaped harmful effect, not because the organisms were harmless, but because of efficient protection. This question is, of course, of much less importance in so far as it concerns *C, D* and *E* who were passively immunized by the injection of antitoxin than it is with regard to *A* and *B* who received no such injection. Although it had been known for some time that certain persons had a natural immunity against diphtheria toxin due to the presence of circulating antitoxin in the blood, the methods of testing for this natural immunity were so tedious as to render their application infrequent. It was not until the introduction of a more simplified method by Schick that large series of persons were tested and some knowledge was obtained as to the comparative frequency of natural immunity to diphtheria toxin. The preliminary report of the work done by Schick appeared in 1908 and this was followed by other reports from time to time.[6] Our attention was not directed to his work, however, until the publication of his papers in 1913, some time after the beginning of these experiments; consequently skin tests were not made on the five volunteers to determine the presence or absence of natural immunity prior to inoculation. Tests performed on them some months later, however, showed that four (*A, C, D* and *E*) had some amount of circulating antitoxin in the blood, whereas *B* on numerous occasions showed a complete absence of antitoxin as indicated by a strongly positive test at each trial. This lack of natural immunity in *B* was later confirmed by the fact that he developed a frank attack of diphtheria the following year (1914) as the result of experimental inoculation with virulent diphtheria bacilli.

It is the opinion of those with wide experience in the use of the Schick reaction that when natural immunity against diphtheria toxin, as manifested by a negative skin test, is once developed in persons beyond the age of five or six years, this immunity is probably life long.[7] If this impression is correct we may infer that *B* was without natural immunity to diph-

[6] Schick, B.: Kutanreaktion bei Impfung mit Diphtherietoxin. Vorläufige Mittheilung, München. med. Wchnschr., 1908, LV, 504. *Also*, Ueber Diphtheriekutanreaktion. Verhandl. d. Gesellsch. f. Kinderh., Köln, 1908, XXV, 330.

Schick and Novotny, J.: Ueber Diphtheriekutanreaktion beim Meerschweinchen. Ztschr. f. Immunitätsforsch. u. exper. Therap., 1910, Th. 1, Orig., IV, 550.

Schick and Michiels, Jules: Die intrakutanreaktion des Menschen auf Diphtherietoxininjektion als Ausdruck des Schutzkörpergehaltes seines Serums. Festschrift für Kassowitz, Julius Springer, Berlin, 1912, p. 232.

Schick and Magyar, Fritz: Versuche mit intrakutaner Injektion von Diphtherietoxin beim Menschen. Verhandl. d. Gesellsch. f. Kinderh., Münster, 1912, XXIX, 9.

Schick: Spezifische Therapie der Diphtherie. Centralbl. f. Bakteriol., 1913, Div. I, Ref., Suppl., LVII, 16.

Schick and Michiels, Jules: Ueber die Wertbestimmung des Schutzkörpergehaltes menschlichen Serums durch intrakutaner Injektion von Diphtherietoxin beim Menschen. Ztschr. f. Kinderh., 1913, Orig., V, 349.

Schick: Die Diphtherietoxin-Hautreaktion des Menschen als Vorprobe der prophylaktischen Diphtherieheilseruminjektion. München. med. Wchnschr., 1913, LX, 2608.

[7] Personal communication from Dr. W. H. Park, Director of the Research Laboratory of the Department of Health, New York City: "As a general statement one can say that a child developing antitoxin and therefore giving a negative Schick test will remain negative for life. We have, however, followed children in institutions from year to year and found that each year about 2 per cent of those who have given negative tests changed to positive. There is undoubtedly some fluctuation from time to time in the amount of circulating antitoxin and also some difference in the toxin dilution employed for the test in the different years, so that I am not quite sure how much to ascribe to the change in the child, and how much to the technique of the injector and the strength of the toxin used. This is equally true for those who have developed a negative Schick test after artificial immunization by means of toxin-antitoxin injections, as indicated by the results of our observations extending over five years. It should be mentioned, however, that some of these apparent changes from a condition of immunity to one of susceptibility are possibly to be explained by mistakes in the records resulting from the fact that two or more children may bear the same name, thus rendering it difficult to avoid confusion."

theria toxin at the beginning of our work, but although we cannot state with certainty that *B* had no protection and that *A, C, D* and *E* had circulating antitoxin and therefore natural immunity against diphtheria toxin at the time these experiments were begun, we know that this was the case when tested some months later, at a time when all the passive immunity conferred by the antitoxin injected in *C, D* and *E* would have long since disappeared. We do not know when any of these persons developed their immunity. In the case of *D,* we know that he was not immune at the age of five, for at that time he had a severe attack of diphtheria. Moreover, just as we cannot say positively that *A, C, D* and *E* were not immune at the time these experiments were begun, so we are unable to say whether any of them developed their immunity as a result of the experimental inoculation with avirulent diphtheria bacilli. That it did not have this effect in the case of *B,* however, has been indicated. The selection of *C, D* and *E* as control individuals to receive antitoxin, all of whom were immune when tested some months later and who probably had homemade antitoxin circulating in their blood at the time our experiments were begun, was entirely due to chance. That *B,* the only individual in the group who was presumably without natural protection, was chosen as one of the two without artificial protection, was chance also.

The clinical effect of experimental inoculation with non-virulent diphtheria bacilli could, of course, be judged only on the two individuals—*A* and *B*—who did not receive a preliminary injection of antitoxin. Of these, *A* had antitoxin present in his blood when tested at a later date—quite possibly this was present before our experiments began—thus leaving only *B* without natural or artificial protection. That *B* was later susceptible to the effect of diphtheria toxin has been pointed out; that he was susceptible, at the time of our work, to whatever ill effect these non-virulent organisms were capable of producing, is most probable.

Viewed in retrospect two shortcomings are quite apparent in the plan of our experiments. First, only individuals without natural immunity should have been selected for this work, certainly for that portion of the group which was not to receive passive immunization; second, there should have been a larger series of these individuals without natural or passive protection, from which to draw conclusions. Bearing in mind these limitations, such facts as we have are presented.

Careful observation of the five persons forming this group failed to reveal any untoward effect resulting from the carrier state thus artificially established by the inoculation of non-virulent diphtheria bacilli into their throats. More specifically, (1) none of them developed clinical diphtheria; (2) none had any subjective symptoms such as sore throat except when due to the attempts at eradication of the bacilli to be described shortly; (3) none showed any objective signs such as redness of the throat, pharyngitis or tonsillitis except when due to the cause just mentioned.

The daily life of these five persons was not restricted in any way. No one outside of the laboratory knew them to be diphtheria bacillus carriers. No cases of clinical diphtheria developed among their associates.

To determine whether any change had taken place in the bacilli as the result of their residence in the human throat, the organisms were isolated at intervals from the positive cultures obtained from these five individuals and tested culturally, tinctorially and by guinea-pig inoculation. Morphologically the organisms were unchanged and their staining characteristics were the same as before they were introduced into the throat. On cultural tests, also, no difference was observed in the character of the growth on Loeffler's blood serum, plain agar or in bouillon, while the changes in litmus milk and in the tests for fermentation of the various sugars were the same as with the original organisms.

Each time an isolation was made the pure culture was tested for virulence. None of the cultures so tested killed a guinea-pig or even rendered one ill. Thus it will be seen that after months of residence in the human throat (15 months in the case of *C* and *E*) there was no evidence of acquisition of virulence.

The question may be raised as to how we know that the organisms harbored by these carriers and recovered by us from time to time were of the same strain that we originally put into the throats. We do not know that they were, but there were certain facts which made us feel relatively sure on this point. In the first place none of the subjects of these experiments were carriers at the time the bacilli were placed in their throats but all of them became carriers shortly thereafter, so shortly as to leave little doubt concerning the relation of the artificial inoculation to the positive cultures obtained from the five individuals. In the second place, the carrier state, once established, lasted for a long time in all—longer in some than in others—but from each individual regularly positive cultures were obtained over a considerable period. These positive cultures, for the most part, showed not one or two organisms to a slide but myriads, indicating that the diphtheria bacilli were not merely persisting but actually multiplying in the throats. Thirdly, the identity of the strain of organisms recovered from the throats with that originally introduced was strongly suggested by the results of the tests applied to the pure cultures isolated at intervals from each of the individuals in the series. The agreement with the original strain was so definite as to render it highly improbable that these five persons had all gotten rid of the original strain and had all picked up another exactly like it.

A very definite carrier state having been established in all five of the subjects of these experiments, an attempt was made to eradicate the bacilli from the throats of *A* and *B*. The work of Churchman [8] has shown the very definite inhibitory action exerted by Gentian Violet on the growth of many of the Gram-positive organisms *in vitro,* among others *B. diphtheriæ,* and this we have confirmed with the strain (No. 48) used in these experiments and with many other strains, virulent and non-

[8] Churchman, J. W.: The Selective Bactericidal Action of Gentian Violet, Jour. Exp. Med., 1912, XVI, 221.

virulent. Accordingly we endeavored to ascertain the effect *in vivo* of this dye upon *B. diphtheriæ*. An aqueous solution of Gentian Violet was prepared in dilution of 1 to 1000 and with this the nose and throat of *A* and *B* were thoroughly sprayed on the evening of March 13, 1913. Within an hour there developed in both individuals the symptoms of an acute coryza. The mucous membrane of nose and throat became congested and there was experienced the raw, burning sensation which characterizes an inflammation of these surfaces. Headache and pain behind the eyes followed and the backache and joint pains of grippe were present. This condition gradually wore off but persisted in less pronounced degree for several days, thus discouraging the further use of an aqueous solution of the dye in a strength of 1 to 1000. The cultures from *A* and *B*, moreover, continued regularly positive. Three days later, after a partial recovery from the discomfort of the first application of Gentian Violet, the treatment was resumed, a more dilute solution, 1 to 3000, however, being employed. The nose and throat were sprayed night and morning for two days with a return of the same unpleasant symptoms which had followed its first use. Two days later (March 31) positive cultures were obtained from both *A* and *B*; in the succeeding eight days, six positive and three negative cultures were obtained. The cultures from *C, D* and *E*, who had not been treated with Gentian Violet, remained regularly positive.

At this time we made a dilution of the dye in normal salt solution in 1 to 10,000 strength and began using it daily on all members of the group, spraying the nose and throat just after the daily culture; this was continued for about ten days. After the inauguration of this treatment:

(1) *A* had eight positive cultures (March 31; April 1, 2 and 17; May 8, 13, 15 and 23) and 14 negative cultures (April 22, 23, 24 and 28; May 1, 9, 12, 20, 22, 24 and 27; June 17 and 26; July 1).

(2) *B* had seven positive cultures (March 31; April 1, 3, 4, 7 and 17; May 6) and 39 negative cultures, 17 prior to his last positive on May 6 and 22 from this date to the time of the end of the series of observations on July 9.

(3) *C* continued regularly positive until after July 1.

(4) *D* continued regularly positive until after April 28, at which time he left the city.

(5) *E* continued regularly positive, with the exception of one negative culture obtained on April 11, until after May 5. The majority of his cultures after this date were positive but from time to time a negative culture was obtained (14 positive and 7 negative from May 5 to July 9).

The results obtained on cultures of these individuals in the succeeding fall, winter and spring will be recalled; *C, D* and *E* continued to be carriers for many months; *A* and *B* did not.

Although the figures might seem to indicate that the Gentian Violet may have had some effect in eradicating the bacilli from the throats of *A* and *B*, the discomfort arising from the use of the more concentrated solutions was so great that the procedure could hardly become popular. It is, moreover, quite possible that the apparent disappearance of the bacilli at about this time was spontaneous and merely coincident with the use of Gentian Violet. It should be pointed out, also, that while occasional negative cultures were first obtained from both *A* and *B* following the employment of Gentian Violet, positive cultures from each of them were encountered at intervals thereafter over a long period (71 days in the case of *A*; 54 days in the case of *B*).

There was no evidence that the use of 1 to 10,000 dilution of Gentian Violet sprayed into the throat once daily had any effect in causing the disappearance of diphtheria bacilli from the throats of carriers.

The two chief difficulties in the use of Gentian Violet for this purposes are (1) its irritating effect upon the mucous membranes and (2) its lack of penetration, the latter, perhaps, depending to a certain extent upon the former. When applied to the throat, there is produced almost at once an abundant secretion of mucus which intervenes between the tissues and the dye, lifting the latter from the surface and preventing penetration.[9]

From the results of some subsequent work—to be reported in another paper—concerning the actual location of the diphtheria bacilli in the throats of carriers, we should not expect that the eradication of these organisms could be accomplished by the use of Gentian Violet or any other substance introduced into the throat by means of swabs, sprays or gargles.

With regard to the symptoms experienced by *A* and *B* following the use of too concentrated solutions of Gentian Violet we feel very definitely that these were not caused by the diphtheria bacilli in the throat. The 1 to 1000 and 1 to 3000 solutions were very irritating and the irritating effect was experienced right after the application and did not recur except when a strong solution was again employed as a spray. The diphtheria bacilli, it is true, were present in the throat at the time, but they had been there from three to four weeks before any symptoms developed and remained for six to ten weeks after the symptoms subsided without causing any difficulty whatever, either objective or subjective.[10] *C, D* and *E*, who had no applications of the stronger solutions, the use of which was followed by unpleasant results in *A* and *B*, had neither symptoms nor local signs in the throat although they were carriers for many months.

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[9] That similar observations have been made by others is seen from the following quotation concerning the treatment of a diphtheria carrier with Gentian Violet. "The results were somewhat suggestive but an unexpected obstacle was met in the secretion of the mucous glands of the pharynx in a thin slimy layer, which was renewed by fresh secretion as rapidly as it was removed and which prevented intimate contact between dye and mucous membrane as effectually as a layer of grease would have done." Churchman, J. W., and Herz, L. F.: The Toxicity of Gentian Violet and its Fate in the Animal Body. Jour. Exp. Med., 1913, XVIII, 579.

[10] Other conceivable but improbable explanations of the symptoms produced by the stronger solutions of Gentian Violet might be mentioned:

(1) Destruction of some of the avirulent diphtheria bacilli by the Gentian Violet, with the liberation of an endotoxin capable of producing the symptoms. (2) Disturbance in a balanced, antagonistic flora produced by Gentian Violet, with production of symptoms by the unopposed group.

SUMMARY

From the results of the experiments herein reported certain facts seem sufficiently important to warrant repetition for the sake of emphasis.

(1) The carrier state was easily produced in human beings by inoculation of the throat with avirulent diphtheria bacilli.

(2) When thus produced the carrier state lasted for a long time; two of the carriers still harbored avirulent diphtheria bacilli after 15 months.

(3) The previous administration of diphtheria antitoxin subcutaneously did not prevent the lodgment and growth of the organisms.

(4) Inoculation of avirulent diphtheria bacilli into the throats of human beings did not produce: (a) clinical diphtheria; (b) any subjective symptoms; (c) any objective change in the appearance of the throat.

(5) The results of the guinea-pig test for virulence were confirmed when thus tested with human beings.

(6) No cases of clinical diphtheria developed among the associates of these artificially produced "healthy carriers" of avirulent diphtheria bacilli.

(7) When isolated in pure culture after prolonged sojourn in the human throat, the bacilli were not altered in morphology or in their staining or cultural characteristics.

(8) The bacilli showed no tendency to become virulent as a result of this type of animal passage, either in the carriers who had received diphtheria antitoxin or in those who had not.

(9) Spraying the nose and throat with Gentian Violet in a strength which could be tolerated seemed to be without effect in eradicating avirulent diphtheria bacilli.

The results of this experimental inoculation of the throats of human beings with avirulent diphtheria bacilli are in entire accord with those obtained in our previous work with healthy diphtheria bacillus carriers. The same general conclusions drawn from our earlier work have merely been confirmed and somewhat amplified.

CONCLUSIONS

1. Avirulent diphtheria bacilli retain their characteristics despite long residence in the human throat or transfer from one human being to another.

2. Avirulent diphtheria bacilli are devoid of pathogenic importance for man.

3. The carrier of avirulent diphtheria bacilli does not constitute a menace to the health of the community.

A CLINICAL METHOD FOR THE QUANTITATIVE DETERMINATION OF CALCIUM AND MAGNESIUM IN SMALL AMOUNTS OF SERUM OR PLASMA

By Benjamin Kramer and Frederick F. Tisdall

(From the Department of Pediatrics, The Johns Hopkins University, Baltimore)

The McCrudden method for the quantitative determination of calcium and magnesium in biological material[1] is considered the most reliable procedure for this purpose. The material to be analyzed is ashed and then dissolved in dilute hydrochloric acid. The solution is heated to boiling and the calcium precipitated by adding an excess of oxalic acid to the acid solution. It is then cooled and the acidity of the solution is diminished by the addition of a large amount of 20 per cent sodium acetate, thereby neutralizing the mineral acid and replacing it with a buffer solution of free acetic acid and sodium acetate in which calcium oxalate is practically insoluble. In the original description of the method the final determination is made by ashing the calcium oxalate until it is completely converted into calcium oxide. The latter is then weighed. The oxalate may, however, be titrated directly by means of acid potassium permanganate.

The latter procedure is to be preferred when determining calcium in small amounts of blood, serum or plasma, since one is here dealing with quantities of calcium too small to be weighed accurately. Under these conditions the amount of calcium oxalate has also been determined colorimetrically or by means of the nephelometer. Of such methods several have been reported. Most of these have been discussed elsewhere and hence will not be reviewed here.[2] All have one disadvantage in common, namely that the protein of the serum must either be removed by a preliminary precipitation or the organic matter destroyed by ashing.

We have succeeded in precipitating calcium and magnesium quantitatively from unashed serum by means of a modified McCrudden technique. This precipitation is the basis of the methods described below for the quantitative estimation of these elements in serum or plasma.

The Sample to be Analyzed

The method may be applied to serum or plasma. If sodium citrate in 0.9 per cent sodium chloride be used to prevent clotting, its final concentration in the blood should not exceed 1.5 per cent. Samples showing moderate or marked hemolysis cannot be used.* Although we have obtained good results

* The addition of oxalic acid or ammonium oxalate to a very dilute solution of ferric chloride yields a precipitate which is only slightly soluble even in glacial acetic acid.

To determine the calcium content of hemolyzed serum dilute 2 c. c. of serum with 2 c. c. of water, then add 1 c. c. of 30 per cent ammonium acetate. After one hour make the volume up to 6 c. c. Centrifuge for 15 minutes, syphon off the supernatant fluid. Use 5 c. c. of this and proceed as for non-hemolyzed serum or plasma. Since the calcium determination is performed on 5/6 of 2 c. c. of serum, the proper correction should be made in the final calculation.

with several sera analyzed at intervals over a period of two weeks, it is preferable that the determination be done within 24 hours. Blood may be obtained by venipuncture or by the Blackfan method.[3] The clotted blood is centrifuged and the serum poured or pipetted off.

The Calcium Method

One or two cubic centimeters of serum or plasma are measured into an ordinary 15 c. c. graduated centrifuge tube containing from 2 to 3 c. c. of distilled water. The tube should be gently agitated after the addition of each drop of serum. Two drops of .01 per cent phenolsulphonphthalein are added followed by 1 drop of n/1 sulphuric acid. One drop of 30 per cent ammonium chloride is then added followed by 1 c. c. of approximately n/1 oxalic acid. The sample should be shaken after each addition.* One-half of 1 c. c. of a saturated solution of sodium acetate is added and the tube allowed to stand for at least one hour. The pH of the sample at this point is about 5.4 (di-brom-cresol). The sample is then made up to a definite volume, preferably 6 c. c., and centrifuged for at least 20 minutes at 1300 revolutions per minute. All but 0.2 c. c. or 0.3 c. c. of the supernatant fluid is syphoned off through a glass tube, the lower end of which is drawn out to a bore of about 1 mm. and curved so that the opening is directed upward. The lower opening in this tube should be at least 3 or 4 mm. above the precipitate. The precipitate is suspended in the residual liquid by stirring with a glass rod. Enough 2 per cent ammonia (2 c. c. of concentrated ammonia diluted to 100 c. c.) is then added to bring the volume to 4 c. c., care being taken to wash the rod and the sides of the centrifuge tube free from adherent oxalic acid. The tube is then centrifuged for 10 minutes. This procedure is repeated twice, thus making three washings in all. After the third washing the supernatant fluid is syphoned off, the tube is shaken to suspend the precipitate, 2 c. c. of approximately n/1 sulphuric acid are added and the tube is warmed in the boiling water-bath for a few minutes and titrated with n/100 potassium permanganate until a definite pink color persists for at least 1 minute when viewed under a good light against a white background. The strength of the permanganate solution is determined by titrating against an n/100 sodium oxalate (Sörensen).

Calculation.—The number of cubic centimeters of n/100 potassium permanganate used diminished by 0.02 c. c. (the blank) times 0.2 equals the mgms. of calcium in the sample. If 2 c. c. of serum are used, this figure multiplied by 50 equals the number of mgms. per 100 c. c. of serum. Thus, if the titration is 1.02 c. c. of n/100 potassium permanganate then $(1.02-.02)\times 0.2\times 50=10$ mgms. calcium per 100 c. c. serum.

Preparation of Reagents.—n/100 sodium oxalate (Sörensen). This is the only reagent that must be quantitatively accurate. An n/10 sodium oxalate (Sörensen) is prepared in the usual way. Six and seventh-tenths grams of sodium oxalate (Sörensen) are dissolved in water. Solution is facilitated by the addition of 5 c. c. of concentrated sulphuric acid and the volume made up to 1 liter. This is diluted ten times to make an n/100 solution. The former solution will keep indefinitely while the latter has been found still unchanged after the lapse of two months.

Approximately n/1 oxalic acid is prepared by dissolving 63 grams of oxalic acid (Kahlbaum or J. T. Baker, C. P.—calcium free) in a liter of water. The acid need be weighed only roughly.

Approximately n/1 Sulphuric Acid.—Fifty cubic centimeters of concentrated sulphuric acid (C. P.) are diluted with water to 1 liter.

Thirty Per Cent Ammonium Chloride.—Approximately 30.0 grams of ammonium chloride are dissolved in 100 c. c. of water. A sample of this should be diluted, an excess of ammonium oxalate solution added and the solution allowed to stand in order to rule out the presence of calcium.

Saturated sodium acetate solution is made by adding an excess of the salt to water and allowing it to stand over night. The supernatant fluid is then filtered.

Sodium acetate (J. T. Baker, C. P.) does not contain calcium.

PROTOCOLS

(1) Samples containing 0.236 mgm. of calcium as $CaCl_2$, .041 mgm. of magnesium as $MgSO_4$ and 0.615 mgm. of phosphorus as KH_2PO_4, were treated as above described for the calcium determination except that no oxalic acid was added. No precipitate appeared at the end of 24 hours. The pH of this solution was about $6.0-6.2$ (di-brom-cresol). This indicates that neither calcium phosphate nor NH_4MgPO_4 is precipitated at this pH under the given conditions.

(2) Similar samples without calcium were treated as described in the method. Here again no precipitate appeared, indicating that magnesium oxalate remains in solution under these conditions.

(3) Solutions containing known amounts of calcium, magnesium and either potassium or sodium phosphate in concentrations corresponding to the maximum which we have found in serum were analyzed. The results given in Table I show that quantities of calcium varying from 0.096 mgm. to 0.386 mgm. can be recovered with a maximum error of 5 per cent.

(4) Known amounts of calcium were added to sera in which the calcium content had already been determined. The added calcium was quantitatively recovered (Table II).

(5) A series of parallel determinations on the sera of humans and animals was made by this method and by the method of Kramer and Howland. The results given in Table III show a satisfactory agreement between the two methods.

DISCUSSION

The investigations of Richards[4] and his collaborators have defined the necessary conditions for a quantitative separation of calcium from magnesium and for the exact gravimetric estimation of the former. The dangers in this procedure lie

* The precipitate which forms on the addition of ammonium chloride disappears on adding oxalic acid.

in the possible occlusion of undissociated magnesium oxalate in the calcium oxalate precipitate when precipitation occurs in a neutral solution in the presence of a large excess of highly ionized ammonium oxalate. The degree of occlusion varies with the concentration of undissociated magnesium oxalate.

In a solution of an electrolyte an equilibrium exists, as represented by the Mass law, betwen the undissociated magnesium oxalate and the free magnesium and oxalate ions, thus:

$$\frac{\overset{++}{Mg} \times \overset{=}{C_2O_4}}{MgC_2O_4} = K = \text{a constant.}$$

The maximum concentration of free magnesium and oxalate ions is determined by the solubility product constant of this salt.[5] This is the product of the ionic concentration of these two ions in a saturated solution of the salt at a given temperature. Thus, if the molar concentration of magnesium oxalate in a saturated solution is .0027 gm. mol. per liter, then if this is 80 per cent dissociated we have the concentration of $\overset{++}{Mg} = .0022$ gm. mol. and that of oxalate $= .0022$ gm. mol., and the solubility product would then be .0000484 or 4.8×10^{-5} gm. mols. per liter.

The addition of a solution which contains a greater concentration of oxalate ions to a saturated solution of magnesium oxalate would so increase the concentration of this ion as to exceed the solubility product constant.[5] The concentration of free magnesium ions is therefore decreased through the formation of more undissociated magnesium oxalate. But the solution is already saturated as regards molecular magnesium oxalate, hence the excess of undissociated magnesium oxalate precipitates. The concentration of magnesium in serum is too small for even a great excess of oxalate ion to bring about precipitation but such an excess can so increase the concentration of molecular magnesium oxalate as to favor its occlusion by calcium oxalate.[6] The dilution of the serum and the addition of a mineral acid counteracts this tendency, the former by decreasing the concentration of both ions, per unit volume, the latter by decreasing that of the free oxalate ion, an effect to be attributed to the large excess of $\overset{+}{H}$ ions which the mineral acid contributes, thereby repressing the ionization of oxalic acid.[6] The mineral acid also keeps calcium phosphate in solution by forming the more soluble acid calcium phosphate ($CaHPO_4$).

McCrudden[1] has shown that in the separation of calcium from solutions containing this element along with magnesium and phosphates the precipitate of calcium oxalate may be contaminated with phosphates, presumably ammonium magnesium phosphate or tertiary calcium phosphate, unless the solution is sufficiently acid during precipitation. The required H-ion concentration is not stated. The proper degree of acidity for complete precipitation of pure calcium oxalate can be best obtained by the addition of a concentrated solution of sodium acetate. We have found that solutions whose pH's vary from 5.2–6.6 will permit complete precipitation of calcium oxalate if sufficient excess of oxalate ions be present.

CONCLUSIONS

I. A method has been described by means of which calcium may be determined directly in 2 c. c. of serum or plasma with a maximum error of ± 5 per cent.

II. No special apparatus is required, except an accurately calibrated 2 c. c. pipette and a micro-burette graduated in 1/50 of a c. c.

III. A determination may be completed within 2 hours after the sample of blood is obtained.

IV. The sources of error in the determination of calcium in small quantities of biological fluids have been discussed.

V. Comparative studies using this rapid method and the method of Kramer and Howland have shown that the former method yields results that agree well with those obtained by the latter.

The Magnesium Method

In 1871 Pribram[7] precipitated completely calcium as calcium oxalate and magnesium as ammonium magnesium phosphate from 100 c. c. samples of serum which had been previously rendered alkaline with ammonia, without preliminary destruction of the organic matter. Gerlach and E. Drechsel[8] stated that this precipitate contains phosphates unless precipitation is accomplished in acid solution. McCrudden[1] demonstrated that fairly accurate results can be obtained when magnesium is precipitated in the filtrate from the calcium determination without oxidation of the organic substances with nitric acid. We have obtained excellent results when omitting this step. Marriott and Howland[9] described a method for the determination of magnesium in small quantities of serum. The essential features of this method are first, the oxidation of the organic matter in the filtrate derived from the calcium determination, second, the precipitation of magnesium as NH_4MgPO_4 by the addition of $(NH_4)_2HPO_4$ and concentrated ammonia, third, the separation of the NH_4MgPO_4 from the supernatant fluid by centrifuging and washing five times, and fourth, the comparison of the degree of decolorization of a solution of Fe $(SCN)_3$ with the decolorization produced by a known amount of NH_4MgPO_4. We have simplified this method by eliminating the first step entirely, by using only one-fifth the amount of $(NH_4)_2HPO_4$, and by filtering the precipitate through a Gooch crucible and washing thoroughly. The colorimetric determination was not changed. The results obtained by the procedure as outlined above are more constant than those obtained by the method of Marriott and Howland.

PREPARATION OF REAGENTS

(1) *Ammonium Magnesium Phosphate Standard.*—This solution is made by dissolving 0.102 gm. of air-dried magnesium ammonium phosphate ($MgNH_4PO_4.6H_2O$) in 100 c. c. of 0.1 N hydrochloric acid and diluting to 1 liter with water. Of this solution 1 c. c. is equivalent to 0.01 mg. of magnesium. Magnesium ammonium phosphate loses water of crystallization when heated and must therefore be dried at room temperature. Commercial preparations of the salt are generally

unreliable; it should be prepared by precipitation of pure solutions. (See Jones, W., J. Biol. Chem., 1916, XXV, 87.)

(2) *Ammonium phosphate solution* is made as follows: 25 gm. $(NH_4)_2HPO_4$ are dissolved in 250 c. c. H_2O. 25 c. c. of concentrated ammonia are added and the mixture is allowed to stand over night. The following day, it is filtered, the filtrate is boiled to remove the excess of ammonia, cooled, and made up to 250 c. c. This solution is diluted 5 times with water.

(3) The *ferric thiocyanate solution* is made from two solutions which are mixed an hour before use. Solution A is 0.3 per cent ammonium thiocyanate. Solution B is 0.3 per cent ferric chloride, made up from the salt with its contained water of crystallization, adding a few drops of acid, if necessary, to clear the solution. Five cubic centimeter portions of Solutions A and B are mixed and the whole is diluted to 40 c. c. with water.

(4) *10% Ammonia.*—One hundred cubic centimeters of concentrated ammonia is diluted to one liter.

THE METHOD

Five cubic centimeters of the supernatant fluid from the calcium determination corresponding to 1.66 c. c. serum is measured into a 30 c. c. beaker, 1 c. c. of $(NH_4)_2HPO_4$ solution is added and then 2 c. c. of concentrated ammonia. Next day the sample is filtered through a well packed Gooch crucible, washed ten times with 5 c. c. of 10 parts of concentrated ammonia to 90 parts of water, then twice with alcohol 95 per cent made alkaline with ammonia. The crucible is returned to the beaker and dried for a few minutes at 80° C. in the oven.

Ten cubic centimeters of n/100 HCl are added to the crucible and after a few hours the entire material is transferred to a test tube, centrifuged, and 5 c. c. of the supernatant fluid is measured into a flat-bottomed colorimeter tube graduated for 10 c. c., which contains 2 c. c. of the iron thiocyanate solution. The volume is then made up to 10 c. c. with n/100 HCl, a rubber stopper inserted and the fluid mixed. A series of standards is prepared by adding varying amounts of a known NH_4MgPO_4 solution to the thiocyanate solution and bringing the volume up to 10 c. c. as in the unknown samples. The color is compared by looking through the entire length of the liquid column against a white background.

Calculation.—The calculation is the same as in the orginal method: Reading (c. c. of standard solution) $\times .01 \times 2 \times 6/5 \times 50 =$ mgms. magnesium in 100 c. c. serum when 2 c. c. of serum are used.

PROTOCOLS

(1) A solution containing an amount of Mg per c. c. as the sulphate corresponding to the amount found in 2 c. c. of serum was analyzed. Magnesium was recovered quantitatively (Table V).

(2) Samples containing Ca, Mg and P in concentrations equal to those found in serum were analyzed both for Mg and Ca and both elements recovered within 5 per cent of the quantity actually present, Table VI.

(3) Magnesium added to serum was quantitatively recovered (Table VII).

(4) A series of determinations on normal adults, normal children, and children suffering from various diseases yielded results that agree well with those found by Marriott and Howland using the more elaborate procedure (Table IV).

CONCLUSIONS

A simplified method for the quantitative determination of magnesium in small quantities of serum or plasma has been described.

TABLE I.—ANALYSES OF SAMPLES OF SOLUTION B

Calcium			
Spec. No.	Found mg.	Present mg.	
1	0.093	....	
2	0.093	....	
3	0.096	....	
4	0.092	....	
Average	0.094	0.096	
5	0.186	....	
6	9.193	....	
7	0.193	....	
8	0.182	....	
Average	0.188	0.192	
9	0.383	....	
10	0.375	....	
11	0.385	....	
12	0.381	....	
Average	0.381	0.386	

Note:

Composition of Solution B

NaCl 4.634 gm.
KH_2PO_4 0.039
$MgSO_4$ 0.758
$CaCO_3$ 0.241
HCl conc. 10.0 c. c.
H_2O to 500 c. c.

TABLE II.—RECOVERY OF CALCIUM ADDED TO SERUM
Authors' Method

	Calcium			
Spec. No.	In serum	Added	Total found	Total present
	mg.	*mg.*	*mg.*	*mg.*
1	0.187	0.096	0.286	0.283
2	0.169	0.236	0.408	0.405
3	0.200	0.236	0.434	0.436
4	0.194	0.236	0.430	0.436

TABLE III.—COMPARATIVE DETERMINATIONS ON SERUM

	Methods	
Spec. No.	Method of Kramer and Howland	Authors' method
1	10.5	10.6
2	10.5	10.5
3	10.6	10.4
4	10.8	10.2

TABLE IV.—CALCIUM AND MAGNESIUM DETERMINATIONS IN NORMAL AND PATHOLOGICAL SERA

Spec. No.	Age	Sex	Diagnosis	Authors' method: Calcium per 100 c. c. serum	Authors' method: Magnesium per 100 c. c. serum
				mg.	mg.
1	Adult.	Male.	Normal............	9.5	2.7
2	"	"	"	10.6	2.9
3	"	"	"	10.3	2.9
4	"	"	"	10.4	...
5	"	"	"	9.2	2.1
6	Sheep.	...	......	9.5	2.1
7	"	...	......	10.0	2.1
8	"	...	......	10.4	1.9
9	17 yrs.	Male.	Osteogenesis imperfecta.	12.1	1.8
10	8 mos.	"	Tetany..........	5.5	1.6
11	8 mos.	"	Tetany (after $CaCl_2$).	7.6	1.8
12	2 yrs.	"	Rickets............	9.2	2.3
13	8 mos.	"	"	7.7	2.2
14	3 yrs.	"	"	7.2	1.8
15	2½ yrs.	Female.	"	9.5	...
16	11 yrs.	Male.	Normal...........	10.2	2.8
17	8 mos.	Female.	Scurvy	10.6	2.2

TABLE V.—ANALYSIS OF SAMPLES OF A SOLUTION OF MAGNESIUM SULPHATE

Spec. No.	Magnesium: Present	Magnesium: Found
	mg.	mg.
1	0.044	0.046
2	0.088	0.092
3	0.044	0.044
4	0.044	0.042

TABLE VI.—ANALYSIS OF 1 C. C. SAMPLES OF A SOLUTION CONTAINING Ca = 0.236 Mg. Mg = 0.041 Mg. P = 0.62 Mg.

Calcium: Found	Calcium: Present	Magnesium: Found	Magnesium: Present
0.230	0.236	0.043	0.044
0.233	....	0.040	....
0.230	....	0.041	....
0.244	....	0.046	....

TABLE VII.—RECOVERY OF MAGNESIUM ADDED TO SERUM

Magnesium: Present	Added	Total found	Total present
mg.	mg.	mg.	mg.
0.038	0.044	0.080	0.082
0.044	0.088	0.137	0.132
0.044	0.044	0.086	0.088
0.042	0.044	0.089	0.086

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THE SURGICAL TREATMENT OF RHINOPHYMA, WITH REPORT OF A CASE

By Edward M. Hanrahan, Jr.

(From the Surgical Department of The Johns Hopkins Hospital)

Two papers on the surgical treatment of rhinophyma have appeared in the current year. Seelig in Surgery, Gynecology and Obstetrics, of April 1920, gave an account of a procedure by which he secured an excellent result. His operation consisted in the excision of the tumor mass and allowing healing to take place by epithelialization proceeding from niduses of epithelium which were left after the main part of the growth had been removed. He states that in the shaving off of the redundant tissue one should bear in mind two things, (1) not to shave so deeply as to remove all niduses of epithelium, and (2) to preserve a thin rim of epithelium around the nares to prevent cicatrization at this point by scar tissue. Bleeding in his case was controlled by pressure. Healing, that is, epithelialization, took place under imbricated strips of sterile zinc oxide adhesive plaster and was complete in ten days. Skin grafting was not found necessary.

Grattan in the Journal of the American Medical Association, May 22, 1920, describes an operation the basis of which is the utilization of skin flaps made by two U-shaped incisions over the tumor. The skin was dissected from the tumor, the mass dissected from the nasal structure and the flaps adapted to the new contour. Healing in his case occurred *per primam* and this patient left the hospital on the 10th day. The problem of improving the appearance of the flaps was met by the application of trichloracetic acid until the higher prominences of the skin were levelled. Roentgen rays were also used subsequently and the result as shown by his illustrations was most satisfactory.

A case of rhinophyma was lately operated on in this clinic by Dr. Mont R. Reid. The technique used was similar to that of Seelig in so far as the entire mass, including the skin, was excised, but differed in that a Thiersch graft was used to cover the denuded surface.

Report of the Case

T. M., a white man, aged 42, was admitted to the hospital on October 8, 1919. The family history was negative. His past history was interesting in the fact that the patient had been troubled with acne vulgaris of the face since boyhood and that he gave also an unusual history of furunculosis. The patient had used alcohol in moderation between the ages of 20 and 40. He had also used chewing tobacco and snuff immoderately.

The first manifestation of the present condition had begun three years before admission during the latter part of 1916. At that time the patient had noticed that his nose was increasing in size. This enlargement had started on the left and spread gradually over the tip of the nose, finally involving the right side. The growth had been very gradual, almost imperceptible, painless and without obstruction to breathing. The patient now sought relief only because of the unsightliness of his appearance. The physical examination was negative except for the condition of the nose and skin. There was a bulbous enlargement of the nose the size of an English walnut, which involved both alæ and the tip. The skin was red, pitted and greasy to the touch. The mass felt firm, not œdematous nor fluctuant. The skin was closely attached to the underlying tumor mass. There was a small wart on the right ala.

The skin of the chest, back and particularly of the forearms was covered with small white scars and comedones. The patient said that the latter became affected only after doing work in which the arms became spattered with oil during the last two years, and that the onset of nasal enlargement had preceded this other condition.

The *Wassermann* was negative. The blood count was practically normal. The blood pressure was 162/105. The urine contained a trace of albumin and a few granular casts. S. G. 1018-1027.

Operation.—October 11, 1919. The nose was sterilized with alcohol. The hypertrophic skin was entirely cut away, healthy skin being left above the line of incision and a border of skin about the nares, about one-eighth of an inch wide. The base was then carefully curetted in order to remove all epithelium, care being taken not to injure the perichondrium. Bleeding was controlled by pressure, hot saline solution and adrenalin, a few catgut 00 ligatures being used on the large vessels. A Thiersch skin graft taken from the thigh was then applied to the denuded area, and a silver foil dressing placed over it. It should be noted that the manipulation during the removal of the tumor expressed large plugs of sebaceous material upon the operative field.

Post-Operative Care.—The recovery was complicated by a short attack of acute tonsillitis during the second week. The nose was dressed on the fifth day. Generally speaking the graft was in good condition except in two small areas on either ala, the larger on the right being about as large as one's little finger-nail. The nose was now kept moist and clean with Dakin's solution applied on compresses moistened every two hours. Healing was complete by the fourteenth day.

Microscopic Study of Excised Tissue.—There is thickening of both layers of the skin—particularly in the stratum corium. There are many papillary downgrowths of epidermis, particularly in the stratum germinativum, with areas of keratinization. The corium is extensively infiltrated with small round cells and shows a great increase of fibrous connective tissue. The ducts of the sebaceous glands are dilated and are lined with thickened epidermis. The glands are dilated and hypertrophied. The corium measures about 1 cm. in thickness and is of the same character throughout as described above. The thickened squamous epithelium of the ducts is present deep in the corium.

The microscopic pathology of this condition was fully described by Wende and Bentz in 1904. They noted the cystic enlargement of the sebaceous glands due to dilatation and hypertrophy of the excretory duct, the connective-tissue hypertrophy and round-cell infiltration and also the hypertrophy and cellular infiltration of the superficial layer of the epidermis. They described the stratum mucosum (germinatum) as thickened, with proliferation of some of the edges.

From a study of the microscopical sections it would seem that the disease is a mild chronic inflammatory reaction brought about by the accumulation of large quantities of sebum in dilated and hypertrophied glands. This inflammatory reaction manifests itself by hypertrophy of connective tissue and

round-cell infiltration in the corium and to a lesser extent is seen in the epidermis—particularly in the germinativum. This being the case, it would seem that the most logical procedure would be the removal of this diseased tissue in its entirety. This is the method employed by Warren and White, and advocated by Seelig and Gibbon who remove all but small niduses of epithelium from which epithelialization proceeds. Grattan leaves only the outer layer of the skin, relying on his dissection of the skin from the deeper layers to remove all sebaceous gland tissue. It was noted, however, by Dubreuilh that in partial excision of slices and union by suture of the remaining portion the latter usually continued to grow and to produce more hypertrophic tissue.

Reid attempts to remove all diseased tissue, even the niduses of epithelium, with the idea of avoiding the development of retention cysts; and then relies on grafts to cover the defect. Either Reid's method or that of Seelig may be followed safely. The graft of healthy skin appears to be a more logical procedure than the use of flaps of skin which should not be considered healthy, and if one fears any injury to the nasal cartilage due to curetting too deeply, the healing from niduses of epithelium offers a very satisfactory result.

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EXPLANATION OF PLATES

FIGS. 1, 2.—Case of Rhinophyma. Duration of growth three years. Patient's age is 42. Acne vulgaris since boyhood.

FIGS. 3, 4.—Ten (10) days after operation. The Thiersch graft has taken.

FIGS. 5, 6.—Photograph forwarded by the patient four months after operation.

FIG. 7.—Photomicrograph magnified 18 times, showing a large dilated duct lined with squamous epithelium. At × a small nidus of epithelium could be left, and from such points the wound may be epithelialized.

FIG. 8.—Photomicrograph magnified 36 times, round-cell infiltration and overgrowth of fibrous connective tissue are apparent. The hypertrophied glands connecting with obstructed and dilated duct are well shown.

THE ISOLATION OF *B. DYSENTERIÆ* (FLEXNER) FROM THE URINE OF AN INFANT WITH DYSENTERY

By Catherine Creighton, C. E. Wagner and Wilburt C. Davison

(From the Harriet Lane Home of The Johns Hopkins Hospital and the Department of Pediatrics of The Johns Hopkins University, Baltimore, Maryland)

True dysentery bacilli have rarely been found in the urine of patients suffering from dysentery.[1] Fraenkel[2] in a large series of urine cultures in adult cases of dysentery recovered dysentery bacilli in only four instances. In one of these patients *B. typhosus* as well as the dysentery bacillus was recovered from the urine. Hilgers[3] among a total of 82 cases of pyelocystitis found *B. dysenteriæ* in the urine of two patients, the first one being a man with chronic prostatitis and the other a child suffering from enteritis follicularis. Foerster[4] isolated *B. dysenteriæ* in another case of pyelocystitis. Sonne[5] found dysentery bacilli in the urine of a patient convalescent from typhoid fever. The organisms recovered in all of these cases were mannite-fermenting dysentery bacilli of the Flexner group. Organisms resembling but not identical with *B. dysenteriæ* have occasionally been isolated from the urine of individuals with other diseases.[6] Hæmorrhagic vaginitis[7] has also been noted as a complication of dysentery.

Our case illustrates that a typical *B. dysenteriæ* (Flexner) (Tables I and II) may be found in the catheterized urine of a child with bacillary dysentery without vaginitis, pyelitis, cystitis or other urinary complication. The organism was discovered as the result of routine cultures.

Case Report

Female, white, No. 23721, aged 27 months. The onset of diarrhœa was rather gradual. The temperature reached 103° F. and there were from ten to fifteen bloody stools on the third day, passed with pain. After one week the patient began to improve and was admitted to this hospital on the fourteenth day of the disease with the complaint of persistent abdominal pain and dysuria. There were two to three bloody stools a day. All the symptoms subsided two days later.

The *physical examination* was essentially negative; there was no microscopic vaginal discharge.

Laboratory Findings.—W. B. C. 10,400; microscopic examination of a centrifuged specimen of uncatheterized urine on the sixteenth day of the disease showed pus cells in clumps (probably from the vagina), but no pus cells were found in catheterized specimens on the sixteenth and nineteenth days. *B. dysenteriæ* (Flexner) was isolated in pure culture from both of these latter specimens. Three weeks later the catheterized urine was sterile. Stool cultures on admission and on the three following days as well as three weeks later were negative for *B. dysenteriæ*. The absence of dysentery bacilli in the stools does not invalidate the diagnosis of bacillary dysentery, for

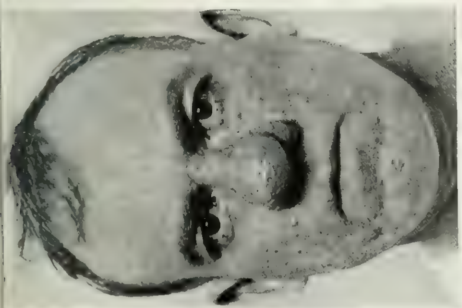
Fig. 1.


Fig. 2.


Fig. 3.


Fig. 4.


Fig. 5.


Fig. 6.


Fig. 7.

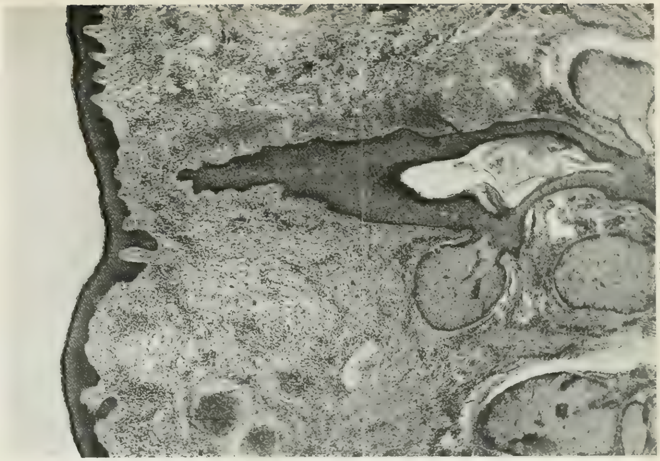
Fig. 8.

stool cultures are frequently negative at this late stage of the disease.

Blood culture one week after admission was sterile.

The agglutination reactions of the patient's serum with cultures of the dysentery bacillus isolated from her urine and also with stock cultures of *B. dysenteriæ* (Flexner) were positive on the seventeenth and thirty-eighth days of the disease (Table III).

Diagnosis.—Bacillary (Flexner) dysentery.

TABLE I.—BIOLOGICAL CHARACTERS OF THE DYSENTERY BACILLUS ISOLATED FROM THE PATIENT'S URINE

Gram stain	Motility	Indol	Gelatin	Lactose	Dextrose	Mannite	Maltose	Saccharose	Dulcite	Rhamnose	Xylose
0	0	0	0	0	+	+	+ (4th) (day)	0	0	0	0

0 indicates negative Gram-staining ; non-motility ; non-formation of indol (8 days) ; non-liquefaction of gelatin (28 days) ; non-fermentation of carbohydrate media (28 days).
+ indicates production of acid without gas in carbohydrate media. For cultural and serological technique, see reference.[6]

TABLE II.—AGGLUTINATION REACTIONS OF THE DYSENTERY BACILLUS ISOLATED FROM THE PATIENT'S URINE

Polyvalent dysentery serum (Rockefeller Institute)	Shiga serum	Polyvalent Flexner serum (Rockefeller Institute)	Flexner diagnostic type sera (Murray)[9]				
			V	W	X	Y	Z
+ 1250	0 100	+ 1000	0 100	+ 252	0 100	+ 250	0 100

+ in Tables II and III indicates complete agglutination at the dilution (end-point) noted after the sign.
0 indicates no agglutination at the dilution noted after the sign.

TABLE III.—AGGLUTINATION REACTIONS OF THE PATIENT'S SERUM

Day of the disease	Cultures of dysentery bacillus isolated from patient's urine	Formolized standard stock cultures					
		B. dysenteriæ (Shiga)	B. dysenteriæ (Flexner) Murray's Types[9]				
			V	W	X	Y	Z
17	+ 100	0 20	+ 20	+ 20	0 20	+ 200	+ 20
38	+ 50	0 20	+ 100	+ 50	0 20	+ 200	+ 20

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A CONTRIBUTION TO THE STAINING OF PHAGOCYTES AND EXUDATES

By Howard B. Cross

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The object of this communication is to place on record the formula of a stain which has been used in this laboratory throughout the last year for the study of phagocytosis and exudates. The preparation and application of this stain are so simple and rapid, the results so uniformly successful, that it seems generally a more satisfactory stain than those commonly used for staining phagocytic preparations. In composition the stain is somewhat similar to the one originally employed by Pappenheim[1] for staining plasma cells. The stain described in this paper, however, has been preferred because with it the nuclei and bacteria are more sharply and constantly stained, annoying precipitates are generally absent, while the uniformly shaded cytoplasm makes possible a clear definition of the cell configuration, and permits a rapid and accurate enumeration of even the smaller and most irregular organisms.

[1] Pappenheim, Arch. f. path. Anat., Berl., 1899, 157, 19-76.

Preparation of Stain.—The following solution is made up using neutral distilled water:

Distilled water (neutral)	100 c. c.
Glycerine	20 c. c.
Alcohol 95%	20 c. c.
Phenol	2 c. c.

To this is then added:

Crystal violet	0.060 gr.
Pyronin	0.200 gr.

The flask is agitated to insure complete solution. This requires not more than two or three minutes. The stain is not filtered and is ready for immediate use. It may be preserved indefinitely if direct sunlight is avoided and the container is kept tightly closed to prevent evaporation of the alcohol.

Method of Using.—The smears of the material to be examined are made upon well cleaned glassware and allowed

to dry in the air. Any further fixation is only an unnecessary addition to the technique without any compensatory advantage. Smears prepared in this manner stick well and if the slides are slightly warmed before the smears are applied it is often possible to preserve the ameboid form of the cells. The slide is then flooded with the stain and after five to ten seconds is thoroughly washed in a jet of distilled water. As direct blotting often injures the cells, it has been found desirable to withdraw the water that may adhere to the smear by touching the side of the slide with a blotter. Stains made in this fashion have been kept in the laboratory for eight months without any marked alterations in the appearance of the preparations.

The best results are possible only when the slides are thoroughly cleaned. It is quite impossible to make a satisfactory smear upon an unclean slide. The presence of grease and other foreign substance presents a precipitating focus which often makes it necessary to discard the smear altogether. Stitt's suggestion of passing the slide slowly through a flame to remove grease and lint before making the smear has proved exceedingly valuable.

Stains made with the above technique present about the following appearance. The cell nuclei are stained violet, the cytoplasm a uniformly delicate lavender. Often a reticular structure can be made out in the extranuclear substance but granules are never present to interfere with the bacterial enumeration. The cell limits are clearly and sharply defined. The bacteria are colored a deep purple and can readily be distinguished from detached nuclear material that may resemble them morphologically. When, however, the intracellular organisms are being digested they gradually lose their staining qualities, appearing as shadowed outlines, and finally disappear leaving only a vacuole filled with granules. Bacteria such as gonococci, which vigorously resist digestion, retain their staining characteristics for days after being ingested. Erythrocytes, when present, appear as pale lavender shadows. Plasma and mast cells exhibit a characteristic structure and stain darkly throughout, so they are at once recognized. Unformed albuminous and mucoid extracellular constituents of exudates seem to possess little affinity for the stain so that the field appears quite clear. There is a total absence of the annoying precipitates too often associated with the staining of phagocytes.

The above stain has also been used as a routine in this laboratory for the preliminary examination of exudates. Its sharp bacterial staining qualities together with the slight affinity of the stain for the extracellular material make possible the demonstration of organisms even when they are present only in exceedingly small numbers. The use of this stain has been found most advantageous in exudates where an excess of cellular debris and serum produce precipitation with resulting confusion when the more common bacterial stains are used. The exact demarcation of cell outlines enables one to determine at a glance the presence of intracellular organisms.

A CLINICAL STUDY OF TUBERCULOUS SALPINGITIS,* BASED UPON 200 CASES

By J. P. Greenberg

(From the Gynecological Clinic of The Johns Hopkins Hospital)

Tuberculosis of the female reproductive organs was formerly considered a very rare condition. Up till the time that Hegar's monumental monograph appeared in 1886 tuberculosis of the female productive organs had been looked upon as a rarity. After the appearance of Hegar's communication, interest in the subject was aroused and since then an enormous literature has appeared. The actual incidence of the disease is still underrated, and there is little wonder that many cases are overlooked when we stop to reflect that the diagnosis is in the majority of cases made only after careful microscopical examination. As recently as 1909 the disease was considered uncommon enough to justify the reports in the literature of individual uncomplicated cases.

In discussing tuberculosis of the tubes, we are really considering the large majority of the cases of tuberculosis of the genital tract, for in nearly all the latter cases the tubes are involved. Furthermore, in a discourse on tuberculous salpingitis we must of necessity make constant reference to tuberculous peritonitis, for the two cannot be separated.

* The complete article, of which this is an abstract, will appear in The Johns Hopkins Hospital Reports.

Incidence.—We have analysed all the cases of tuberculous salpingitis which have occurred on the Gynecological service of The Johns Hopkins Hospital during the last 30 years, (1890-1919 inclusive). In this report we shall give a clinical study of those cases only which showed definitely, upon microscopic examination, that tuberculosis was present. Fulfilling this requirement we found exactly 200 cases. This, of course does not give the actual morbidity of the disease; for many patients having tuberculous peritonitis with tubal involvement did not have their tubes removed. We had 67 such patients; and if we agree with Osler and Menge that about 35% of these patients have tuberculous salpingitis, we find that among the 67 patients, 23 had tubal tuberculosis. Hence instead of 200 cases of tuberculous salpingitis we have had in all probability 223. Our clinical study, however, is based only on the 200 cases in which the tubes were without doubt tuberculous.

During the 30 years there have been 24,155 patients suffering from gynecological disorders. The incidence of tuberculous salpingitis therefore is 0.83%, or considering the number of patients as 223, we find that the incidence is 0.92%.

Incidence of Tuberculous Salpingitis Compared with All Diseases of the Tubes.—During the last 30 years there have

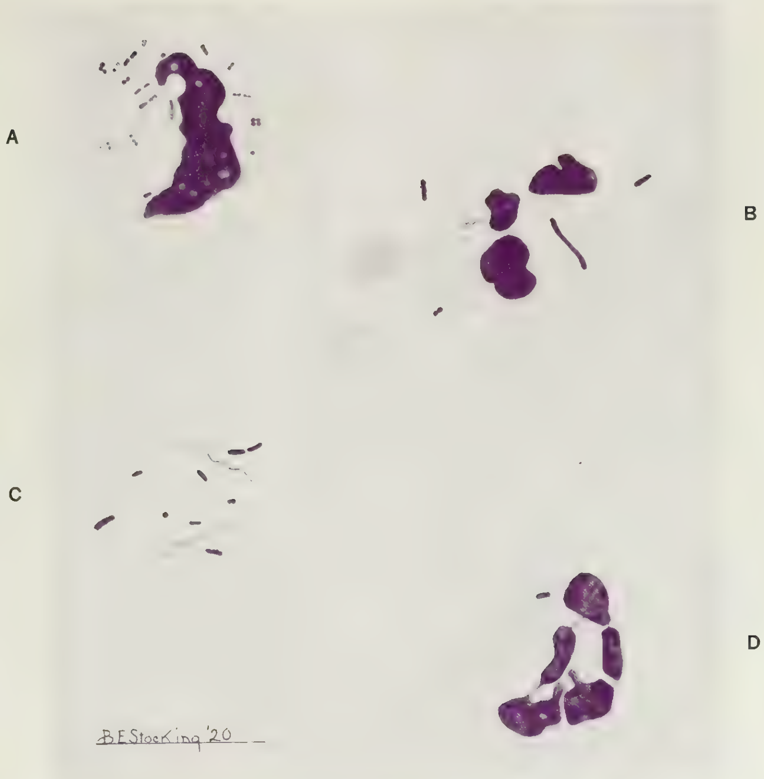

FIG. 1. Illustrating the appearance of phagocytes and bacteria in smears prepared according to the technique described in this paper. (A) Pus cell from a lung abscess in a multiple infection. (B) Polymorphonuclear leucocyte from the blood of a guinea-pig twenty five minutes after the ingestion of B. proteus. The bacteria are surrounded by vacuoles. One bacillus, partially digested, has lost its staining characteristics. The cell is associated with erythrocytes. (C) Smear from a lung abscess containing cocci, bacilli, and spirochetes. (D) Polymorphonuclear leucocyte containing a colon bacillus within a digestive vacuole.

been 3382 salpingectomies at The Johns Hopkins Hospital. Of this number, in 423 the tubes proved to be normal. There remained then 2959 cases in which the tubes were pathological. Since there were in this period of time 200 cases of tubal tuberculosis, the incidence of tuberculous salpingitis as compared with the abnormal tubes is 6.76%. This figure however does not give us the actual incidence. We must take into consideration the 23 cases before mentioned, in which there was peritoneal tuberculosis but in which the diagnosis of tubal tuberculosis was not verified because the tubes were not removed. Considering therefore 223 cases, we find that of the 2959 pathological tubes, 7.5% were tuberculous, a figure that more nearly approximates the truth. This means that out of every 13 abnormal tubes removed, one was tuberculous.

Age.—Our youngest patient was 14 years old; while the oldest was 55. The greater number (73.5%) were in the child-bearing period (between 20-40 years).

Fertility.—In our series there were 159 (79.5%) married and 41 (20.5%) unmarried women. Of the former, 101 (63.5%) had never been pregnant, and since about 90% of them had been married at least two years, which may be accepted as a reasonable period for the determination of the sterility or fertility of a married woman, we may assume that about 60% of our patients were actually sterile. Of the 41 single women, six had been pregnant (14.6%).

Symptoms.—The various symptoms are considered in full in the complete report. We shall here briefly refer only to those of major importance.

On admission to the hospital, more than 80% of the patients complained of pain in the lower abdomen, and about 30% had concomitant pain in the back. Pain around the umbilicus, which is so often quoted as being characteristic of tuberculous peritonitis, was elicited in only 7%, although, as will be pointed out, 63% of the patients had peritoneal tuberculosis.

The following menstrual disturbances were noted; dysmenorrhea (62%), menorrhagia (41.5%), oligomenorrhea (24%) and amenorrhea (6.5%).

Among the 200 patients, 72% had a leukorrheal discharge and all the patients with tuberculosis of the cervix had leukorrhea.

Predisposing Causes.—An appreciable number of patients attributed the onset of their infection to some special uterine activity. Seventy-four patients gave a history of a definite onset, and of this number 46 or 62.2% (23% of the 200 patients), dated their trouble from some uterine function or disturbance, as may be seen from the following table:

Time of onset		No.		%.	
Menstrual period	Uterine Activity	22	46	11.0%	23%
Childbirth		15		7.5	
Miscarriage		6		3.0	
Pregnancy		2		1.0	
Induced abortion		1		0.5	
Marriage		9		4.5	
Acute illness		9		4.5	
Operation		4		2.0	
Injury		4		2.0	
Menopause		1		0.5	
Fright		1		0.5	
		74		37.0%	

Physical Examination.—For the complete data bearing on the physical examination, the reader is referred to the more extensive article. Mention may here be made, however, of the more important findings.

Pulmonary tuberculosis occurred in about one-fourth, and tenderness in the lower abdomen was elicited in about three-fourths of the patients. Of interest in the pelvic examination was the fact that although 7 patients had tuberculosis of the cervix, the condition was recognized only once. In 70%, masses were found in the fornices, replacing the normal adnexa.

Elevation of Temperature.—Considering a temperature of 99.2° or above as fever, we found that 125 of our 200 patients (62.5%) had a pre-operative elevation of temperature. Among the patients having peritoneal involvement, 65.8% had pre-operative fever, while of those having apparently no peritoneal involvement, it occurred in only 34.2%.

Leukocyte Count.—The leukocyte count was recorded 50 times. Only 34% of these patients had a count of more than 9000, while 22% had an absolute leukopenia. Whereas the remainder apparently had a normal count (between 6000-9000), they in reality had a relative leukopenia. For most of them had fever at the time the count was made and in conditions other than tuberculosis a definite leukocytosis should have been found.

Hemoglobin.—In 62 patients the hemoglobin was determined by the Sahli method. If we consider 70% as the lower normal limit, we find that 50% of these patients presented too low a hemoglobin.

Pre-Operative Diagnosis.—No diagnosis was recorded before operation in 34 instances. The correct diagnosis was made before operation in only 26 instances (13%). However, 15 of these patients had ascites which made the diagnosis an easy one. The most frequent diagnosis was "Pelvic Inflammatory Disease," by which was meant in practically all instances, a gonorrheal or puerperal affection. Myomata were diagnosed 29 times and were actually found associated with tuberculosis in 25 cases.

Treatment.—All the cases but two which are here reported were operative cases and in every instance the operation was performed by the abdominal route. In 10 patients this was combined with pelvic puncture. A radical operation was performed 106 times (53%). The uterus was removed in 124 cases (62%), while there were 166 oöphorectomies (83%), unilateral or bilateral. Salpingectomy alone was performed 18 times (9%). Exploratory laparotomy was done in five cases, in all of which the autopsy showed tuberculous tubes.

Drainage.—Among our 200 cases 104 (52%) were drained. Recourse was had to abdominal drainage in 28 instances (14%), to vaginal drainage 58 times (29%) and to combined abdominal and pelvic drainage in 18 cases (9%). In the 104 patients who had been drained, there were 18 fecal fistulæ, an incidence of 17.3%. None of the 96 patients who were not drained developed a fecal fistula.

Order of Frequency of Organs Involved.—The order of frequency of involvement of the various pelvic organs in the tuberculous process is as follows:

Tubes	200
Uterus	90
Ovaries	59
Cervix	7
Vagina	1

Tuberculosis of the Uterus.—The uterus was involved 90 times (45%). If we seek the incidence of uterine tuberculosis among the hysterectomies, we find it to be 72.6%. This figure more nearly represents the actual occurrence of tuberculosis of the uterus. The following table shows the distribution of the disease process in the uterus:

Endometrium	86
Myometrium	12
Endometrium alone	78
Myometrium alone	4
Endometrium and myometrium	8

As may be seen, tuberculosis limited to the myometrium is rare. Of the 86 patients with tuberculous endometritis, all but three were in the menstruating age and among them amenorrhea occurred in only 7 (8.4%); menorrhagia in 37.2%.

Tuberculosis of the Ovary.—The ovaries were found to be tuberculous 55 times; hence the incidence based upon the number of oöphorectomies (166) was 33.1%.

Tuberculosis of the Cervix.—In our 200 cases of tubal tuberculosis, the cervix was involved seven times (3.5). In addition to these, two other cases of tuberculosis of the cervix were noted but not associated with tuberculosis of the tubes.

Among the cervical cases associated with tubal tuberculosis other portions of the uterus were involved as follows:

Cervix and endometrium together	3
Cervix, endometrium and myometrium	3
Cervix alone	1

Tuberculosis of the Vagina.—Vaginal tuberculosis was found in only one case (0.5%).

Tuberculosis of the Peritoneum and Appendix.—Peritoneal tuberculosis was found in 126 of the 200 patients (63%). In most of our cases the appendix was removed. We found six cases of tuberculosis, making our incidence at least 3%.

Combinations of Organs Involved.—The following list indicates the manner in which the various organs were associated together in the disease.

Tubes involved alone	37	18.5%
Tubes and peritoneum	49	24.5
Tubes and uterus	23	11.5
Tubes, uterus and peritoneum	33	16.5
Tubes and ovaries	3	1.5
Tubes, ovaries and peritoneum	19	9.5
Tubes, uterus and ovaries	8	4.0
Tubes, uterus, ovaries and peritoneum	20	10.0
Tubes, ovaries and cervix	1	0.5
Tubes, ovaries, uterus and cervix	1	0.5
Tubes, uterus and cervix	1	0.5
Tubes, uterus, cervix and peritoneum	1	0.5
Tubes, uterus, ovaries, cervix and peritoneum	3	1.5
Tubes, uterus and vagina	1	0.5
	200	100.0%

Deaths in the Hospital.—In our 200 cases there were 17 deaths in the hospital (8.5%). Of this number, however, only 15 of the patients had been operated upon; hence the operative mortality in 198 cases was 7.6%.

Of the 17 patients who died, 7 had definite signs of pulmonary tuberculosis on physical examination, while in 3 others the diagnosis was questionable. Or, considered differently, 9 of our 58 patients with definite or suspicious phthisis died immediately after operation (15.5%), as compared with 7 (5%) in 142 patients without pulmonary involvement.

Out of our 125 patients with an elevation of temperature, there was 14 deaths (11.2%), while in the 75 patients without fever, there were only three deaths (4%).

In the 126 patients with peritoneal involvement, the operative mortality was 11.1%; in the 74 patients without peritoneal tuberculosis the mortality was only 2.7%.

It appears, therefore, that the prognosis is graver when there is tuberculosis elsewhere, when a pre-operative elevation of temperature exists, and when the peritoneum is involved in the tuberculous process.

Late Results.—An effort was made to communicate with the 183 patients who were discharged from the hospital, but unfortunately information could be obtained only regarding ninety (66 white and 24 colored women). To draw conclusions from these is not only hazardous but is also erroneous, as only half the patients are represented. However, the results are interesting.

Of the 90 patients, 12 had died; but 3 deaths had had no relation to the tuberculosis. (One after a gall-bladder operation, one after an automobile accident and the third following an operation for carcinoma of the abdominal wall.)

Among the 78 living patients who responded, the general health had markedly improved after operation in 73, somewhat in 2 and not at all in 3. There had been a gain in weight in 73 patients, which in many instances was very marked. The pain had been absolutely relieved in 72, partly in 4 and not at all in 2. Eleven patients complained of bladder disturbances. Ten patients who were discharged from the hospital with open wounds, reported that the wounds had subsequently closed, at intervals varying from 2 weeks to 2 years. In 3 patients the wound, which was healed at the time of discharge from the hospital, reopened, but closed again spontaneously. Post-operative hernia occurred in 7 patients.

CONCLUSIONS

We may summarize the findings of our 200 cases of tuberculous salpingitis as follows:

1. Tuberculous salpingitis occurred in nearly 1% of all women admitted on the gynecological service of The Johns Hopkins Hospital.

2. It was found one and a half times as frequently among the colored women as among the white.

3. Out of every 13 abnormal tubes removed at operation, one was tuberculous.

4. Nearly three-fourth of the patients were between 20 and 40 years of age.

5. Sixty per cent of the married patients were sterile.

6. A family history of tuberculosis was reported in 22.5%, while in an additional 2.5% the consort had active pulmonary tuberculosis.

7. The chief complaint of the patient was pain (74.5%) usually situated in the lower abdomen (82.5%).

8. Not much information was obtained from the menstrual history. However, 62% of the patients had dysmenorrhea and 41.5% menorrhagia. Amenorrhea occurred in only 6.5% of the patients.

9. Leukorrhea occurred in 72% of the patients.

10. Nearly half of the patients had dysuria, nycturia and pollakiuria.

11. More than half of the patients were constipated.

12. Approximately one-fourth of the patients attributed the onset of their symptoms to some uterine activity (menstruation, pregnancy, etc.).

13. Half of the patients had lost weight during their illness.

14. The physical examination presented no characteristic findings.

15. About one-fourth of the patients had pulmonary tuberculosis.

16. Pre-operative elevation of temperature was recorded in 62.5%.

17. There was usually either an absolute or a relative leukopenia.

18. Half of the patients examined had a hemoglobin below normal limits.

19. The correct diagnosis before operation was made in only 13% of the cases and, in more than half of these, the diagnosis was aided by the presence of ascites.

20. A radical operation was performed in 53% of the cases.

21. Complications during operation occurred in 14.5% of the patients.

22. One hundred and four cases were drained. Of these, 17.3% developed fecal fistulæ. Abdominal fecal fistulæ occurred 3 times more frequently among the cases drained abdominally than in those drained through the vagina.

23. In one-third of all the patients, there was suppuration of the abdominal incision.

24. No patients who were not drained, developed a fecal fistula.

25. The incidence of fecal fistulæ among the cases in which the bowel had been injured was 48%, whereas among the cases in which the intestines had remained intact, the incidence was only 3.4%. The patients with pre-operative fever developed fecal fistulæ more than twice as frequently as those without fever.

26. Urinary fistulæ occurred 5 times (2.5%).

27. The order of frequency of involvement of the pelvic organs was as follows: tubes, uterus, ovaries, cervix and vagina. Tuberculosis of these organs was found associated with tubal tuberculosis as follows: uterus, 72.6%; ovaries, 33.1%; cervix, 3.5%; and vagina, 0.5%.

28. In 99% of the cases both tubes were involved.

29. In 68% of the cases the peritoneum was involved, and in 3% the appendix was tuberculous.

30. In 17% myomata uteri were associated with the tuberculous process.

31. In only 2% was there an associated involvement of the urinary tract.

32. The prognosis is grave in the presence of tuberculosis elsewhere in the body, where fever exists and where the peritoneum is involved.

33. Our operative mortality was 7.6%. (This includes all the patients who died in the hospital.)

34. By means of follow-up letters, etc., 90 patients were traced and out of this number, 78 were found to be living from 2 months to 30 years after the operations. Nearly all those who are alive are in good condition.

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THE CAUSE OF SO-CALLED IDIOPATHIC HYDROCEPHALUS

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Until recently all cases of hydrocephalus were considered idiopathic. We think it is now fair to assume that those cases in which an obstruction in the ventricular system can be demonstrated may be liberated from this *terra incognita* and may now be classified according to an established pathology; for there can no longer be any doubt concerning the cause of obstructive hydrocephalus. Any lesion which occludes the ventricular system will always produce stasis of fluid and dilatation of the ventricles proximal to the obstruction but will not change the size of the ventricles distal to it. There can be no exception to this rule. The proof of this causative relationship has been amply provided in recent necropsy material [1] and in the experimental production of the disease at will.[1, 3, 4, 5]

[1] Dandy, W. E., and Blackfan, K. D: Internal Hydrocephalus—an Experimental, Clinical and Pathological Study. J. A. M. A., 1913, lxi, 2216; Am. J. Dis. Child., 1914, viii, 406.

[3] Dandy, W. E.: Experimental Hydrocephalus. Ann. Surg., August, 1919, p. 129.

[4] Weed, L. H.: The Experimental Production of an Internal Hydrocephalus. Publication 272, Carnegie Institute of Washington, p. 425.

[5] Thomas, W. T.: Experimental Hydrocephalus. J. Exper. Med., 1914, xix, 106.

The purpose of this paper is to present proof—which I believe is just as positive—of the cause of the remaining big group of this disease—*communicating hydrocephalus, i. e.,* of that type of hydrocephalus in which all the ventricles are in communication with the subarachnoid space. In the course of intensive studies on the absorption of cerebrospinal fluid in hydrocephalus it was found that in the communicating type the absorption from the subarachnoid space was greatly reduced. A reduction in the amount of the absorbing spaces which are reached by the cerebrospinal fluid was suspected as the cause and a hypothetical pathology suggested along this line of reasoning.[1] Later, four cases of communicating hydrocephalus were studied at necropsy and in each adhesions were found which obliterated the cisternæ; hence it was assumed that, by preventing the cerebrospinal fluid from reaching the great absorbing spaces over the cerebral hemispheres, these adhesions had caused the hydrocephalus.[2] We realized, however, the necessity of a more graphic demonstration of the lesion and of proof of its effects before these findings could be accepted beyond question.

[2] Dandy, W. E., and Blackfan, K. D.: Internal Hydrocephalus. Second paper. Am. J. Dis. Child., 1917, xiv, 424.

There are two ways in which an obstruction of the cisternæ can be clearly shown—one after death, the other in the living patient. (1) If a colored suspension is carefully injected into the spinal canal before making an autopsy[3] the color will reach but cannot pass an obstruction in the cisternæ. (2) If air is injected into the spinal canal of a living patient, the roentgenogram will show the air extending up to but not beyond the point of obstruction in the cisternæ.[6,7] Unless these tests are positive, an obstruction in the cisternæ or elsewhere cannot be presumed to exist nor considered to be the cause of communicating hydrocephalus. If properly applied, either test will prove conclusively that an obstruction either is or is not present.

A third and equally important proof must be forthcoming before the cause of communicating hydrocephalus can be regarded as solved: it must be shown that a lesion similar to the one described in these cases of communicating hydrocephalus and similarly situated will cause hydrocephalus when experimentally produced in animals.

Communicating Hydrocephalus Experimentally Produced

In the studies presented here, all of these exactions have been met. First I have produced communicating hydrocephalus in dogs by making a barrier of adhesions in the mesencephalic cisterna[3] (Fig. 1). Shortly before necropsy on these animals a suspension of India ink was substituted for an equal amount of cerebrospinal fluid which had been aspirated from the cisterna magna through a puncture of the occipito-atlantal membrane. When India ink is introduced into the spinal canal of an animal whose cerebrospinal spaces are intact, the color will find its way within two hours to every point of the subarachnoid space over both cerebral hemispheres. But in the experimental animal with the perimesencephalic band of adhesions, the passage of the ink is abruptly terminated by the obstructing band and none of the color reaches the surface of either cerebral hemisphere (Figs. 3, 4); furthermore, as a result of the hydrocephalus which has developed, the foramina of Luschka and Magendie have become so dilated that a retrograde flow of ink is freely permitted into all the cerebral ventricles (Fig. 3). The entrance of ink into the furthermost recesses of the ventricles (which normally occurs only at times) shows that the color has had every opportunity to reach the cerebral sulci, but is precluded from doing so by the obstruction.

Demonstration of Obstruction in the Subarachnoid Space at Post-Mortem Examination

Knowing from the experiments that an obstruction in the cisterna produces communicating hydrocephalus, it then remains to prove that all or at least many cases of this disease have this as the causative lesion. The graphic color method should be applied to all human necropsy material in which hydrocephalus is suspected or known to be present. It is important that pressure be avoided in introducing these colored solutions, for delicate adhesions, though sufficient to cause an obstruction during life, may be easily ruptured and in this way artificial results may be obtained. In animals, the color can be introduced without pressure during life and the normal circulation will convey the fluid to all the spaces which are patent. In necropsy material the results, though less perfect, will be satisfactory if the color is introduced by gravity for 15 or 20 minutes.

Despite studies in a large series of cases of hydrocephalus, we have had but one opportunity of applying this method at a post-mortem examination in a case of hydrocephalus with communication. In this instance the results were just as striking as in the experimental cases which have been described; the color filled the cisternæ, even the cisterna interpeduncularis, covered the cerebellar subarachnoid spaces, but failed to reach any of the sulci over either cerebral hemisphere (Figs. 17, 18, 19). On the other hand, the ink passed freely into every part of the cerebral ventricles, deeply staining their walls (Fig. 18), for both foramina of Luschka and the foramen of Magendie were widely open (Fig. 19). No gross adhesions could be seen either in the vicinity of these openings or even along the cisternæ; nevertheless, the cerebral sulci could not be reached by the colored solution, because the branches issuing from the cisternæ were sealed. The character of the pathological lesion will be discussed later; the test demonstrates that an obstruction exists in the cisternæ and with the additional support of the experimental evidence no doubt can exist that this obstruction is the cause of the hydrocephalus.

Why Should an Obstruction in the Cisternæ Cause Hydrocephalus?

Doubtless obstructions, similar to those which we are about to describe in communicating hydrocephalus, have been present in all necropsies of this disease. The adhesions may not be striking, and at times could be missed entirely, if one did not look for them. Indeed in some cases the lesion may be due to defective formation of the cerebrospinal spaces in the early embryo. These pathological findings become significant and all-important only when the anatomy of the cerebrospinal spaces and the manner and place of the formation and the absorption of cerebrospinal fluid are fully understood.

Cerebrospinal fluid circulates in a mesothelial-lined vascular system which is just as definite as the vascular systems for blood, lymph or bile. A clear conception of the gross plan of this vascular system can be obtained from the accompanying diagram by Max Brödel (Fig. 5). The cavities in the interior of the brain (the ventricular system) are concerned only with the *production* of cerebrospinal fluid; the spaces on the exterior of the brain (the subarachnoid spaces) are normally concerned only with the *absorption* of cerebrospinal fluid. The balance between the production and absorption of fluid is maintained by three closely grouped communicating open-

[6] Dandy, W. E.: Roentgenography of the brain after the injection of air into the spinal canal. Ann. Surg., October, 1919, p. 397.

[7] Dandy, W. E.: Ventriculography following the injection of air into the cerebral ventricles. Ann. Surg., July, 1918, p. 5.

ings—the foramina of Luschka and that of Magendie (Fig. 19). Only through these openings can fluid escape from the entire ventricular system; consequently, closure of these openings always produces a stasis of fluid—hydrocephalus—in all the ventricles. But in communicating hydrocephalus, these conduits are open, either entirely or in part, depending upon the extent and position of the pathological lesion. This type of hydrocephalus is caused by interference with the absorption of the cerebrospinal fluid in the subarachnoid spaces. The real absorbing area of the subarachnoid space is the great network of subarachnoid spaces over the cerebral hemispheres—the cerebral sulci. Here the cerebrospinal fluid is distributed over a very extensive surface of blood capillaries of the pia and passes directly through the capillary walls into the blood by osmosis. Numerous large branches convey the fluid to these spaces from the cisterna chiasmatis and the cisterna interpeduncularis, which together serve as a distributing center for all the cerebrospinal fluid which is destined to reach the cerebral hemisphere. Since all the ventricular fluid, on leaving the ventricles, first reaches the cisterna magna (by way of the foramina of Luschka and Magendie), a relatively long passageway under the medulla, pons, and mid-brain must be traversed before this fluid can reach the cisterna interpeduncularis and the cisterna chiasmatis, whence it can be distributed to the cerebral sulci by the major branches, as described (Fig. 5). The finer anatomy and histology of these spaces have been well described by Weed.[8, 9]

By experimental methods, which have been mentioned in earlier publications, it has been shown that from three-fourths to four-fifths of the cerebrospinal fluid is absorbed from the subarachnoid spaces of the brain, and the remaining one-quarter or one-fifth in the spinal subarachnoid space. It is doubtful if any absorption occurs in the cisternæ, these channels probably serving only as large conduits to carry the fluid to the surface of the brain, much as the ureters carry the urinary secretion to the bladder. An obstruction in the cisternæ under the medulla, pons or mesencephalon (that is, at any point between the foramina of Luschka and the cisterna interpeduncularis) will produce a stasis of fluid up to the point of obstruction and cause hydrocephalus just as effectively as would a block at the aqueduct of Sylvius or at the foramina of Luschka and Magendie. It will be remembered that through the membranous tentorium cerebelli which separates the posterior and middle cranial cavities there is but one opening, and this is only a little larger than the brain stem (mesencephalon) which passes through it. It is evident that when adhesions close the incisura tentorii and obliterate the mesencephalic cisterna, collateral channels for the distribution of cerebrospinal fluid have no possible way to develop.

[8] Weed, L. H.: An Anatomical Consideration of the Cerebrospinal Fluid. Anat. Record, 1917, xii, p. 461.

[9] Weed, L. H.: Cells of the Arachnoid. Bull. Johns Hopkins Hosp., 1920, xxxi, p. 343.

Intra Vitam Method of Demonstrating an Obstruction in the Subarachnoid Space

The value of intraspinous injections of air will be apparent when it is realized that every part of the subarachnoid space can be reproduced[6] in the roentgenogram, just as every part of the ventricular system can be reproduced by an *intraventricular* injection of air.[7] At times, the ventricles also can be injected from the spinal puncture and, again, the subarachnoid space may be partially or wholly injected by way of the ventricular puncture. The patient is placed in the recumbent position, with the head exactly horizontal and higher than the body. This position must be carefully maintained until the skiagram has been taken. In the normal adult, about 30 to 60 c. c. of fluid can be obtained by lumbar puncture and an equal quantity of air, which is substituted, will fill all parts of the subarachnoid space. The cerebral sulci are shown as a network of lines over the brain (Fig. 6). The presence or absence of these air-filled sulci is the crucial observation of all intraspinous injections. Normally, the sulci will always be filled. When they can be seen over the entire cerebral hemisphere, it is evident that every part of the subarachnoid space is patent. Intact subarachnoid spaces may be interpreted to mean that hydrocephalus, if present, cannot be of the communicating type; if, therefore, hydrocephalus is present (with air-filled sulci) an obstruction must be located in the ventricular system. On the other hand, the absence of air in the cerebral sulci means that an obstruction exists in some part of the subarachnoid space; it also indicates that hydrocephalus must exist because the cerebrospinal fluid (air) cannot reach the absorbing spaces of the cerebral hemispheres; the hydrocephalus with such pneumographic findings would be of the communicating type or possibly of an obstructive type, which, if corrected, would only be transformed into a communicating type.[6, 7]

When intact, the cisternal conduit can often be seen throughout the entire course, even through the dense petrous portion of the temporal bone; the major branches can frequently be seen passing directly from the cisterna chiasmatis and the cisterna interpeduncularis to the cerebral sulci; and when an obstruction exists at any point along the cisternæ, it is located definitely by the furthermost point of the air shadow. The cisterna magna is usually clearly outlined. A marked variation has been found in its size; to a certain extent, I believe its size depends upon effects of adhesions, which are so frequently present; for if the cerebellar lobes are firmly bound to the dura in the neighborhood of the foramen magnum, it is clear that the size of the cisterna magna will be reduced and that its enlargement which would otherwise naturally occur with hydrocephalus will be impossible. In obstructive hydrocephalus the size of the cisterna magna is usually reduced by the backward pressure of the superimposed dilated ventricles, the contents of which have no avenue of escape into the cisternæ.

[6, 7] Dandy, W. E.: *Loc. cit.*

Location of Occlusions of the Subarachnoid Space

Obviously an obstruction can exist at any part of the subarachnoid tree and the results, in terms of hydrocephalus, will be dependent upon the location of the obstruction. The obstruction may be in the trunk of the tree (the cisternæ); it may occlude all the main branches which carry fluid from the cisternæ to the cerebral sulci; it may occlude some, but not all, of these branches; or finally, more or less extensive local areas of the subarachnoid space may be obliterated. An obliteration of the cisternæ or of all the distributing branches will prevent any cerebrospinal fluid from reaching any of the cerebral sulci; occlusion of some but not all the branches of the cisternæ may or may not produce hydrocephalus according to the number of cerebral sulci which continue to receive fluid through the branches which remain intact; or a low grade of hydrocephalus may develop because part of the fluid will be handled by the patent sulci. Extensive local areas of the cerebral subarachnoid space may be destroyed without the occurrence of any hydrocephalus, because the normal subarachnoid spaces are far in excess of the normal requirement for absorption; this is demonstrated by the results of every cranial operation, following which adhesions obliterate extensive areas of subarachnoid spaces with no effect upon the balance of cerebrospinal fluid.

Obstruction in the Cisternæ

The most frequent location for an obstruction in communicating hydrocephalus is in the cisternæ. This was first observed in the four cases which were carefully studied at necropsy and will be seen in the results which are to follow in the patients who have been studied by cerebral pneumography. One must not infer from this statement that adhesions only in the cisternæ exist in these cases, but merely that these are the adhesions which are directly responsible for the production of the hydrocephalus. As a matter of fact, there are frequently more or less extensive adhesions along the entire base of the brain, particularly over both cerebellar lobes, and even over the cerebral hemispheres. Frequently one or two of the three basal foramina (Luschka and Magendie) may be sealed by these adhesions (Fig. 12) and at times the lumen of the third opening may be implicated. When all three openings are occluded, obstructive hydrocephalus results; when one or more foramina are patent, the hydrocephalus is of the communicating type.

Certainly the vast majority of all cases of communicating hydrocephalus follow meningitis (Fig. 8) and, being a post-meningitic process, the obstruction of the cisternæ is in keeping with the basilar involvement of most forms of meningitis. It is also worthy of note that the great majority of these cases occur in infants and young children in whom meningitis is so prevalent and in whom the delicate meninges are more susceptible to permanent injury. At times the meningitic process may be of prenatal origin. This is shown by the frequent occurrence of this type of hydrocephalus at birth and by the presence of the basilar adhesions as the etiological factor; also by the coexistence of a meningocele which is doubtless caused by the same general process.

In more than half of our cases of communicating hydrocephalus the disease has definitely arisen at some time after birth, usually following an illness which has been variously diagnosed, but which a careful history will prove to have been meningitis. Again, the hydrocephalus has almost certainly followed an acute illness, perhaps even very mild, but which on the most careful inquiry has yielded none of the signs or symptoms of meningitis. In these cases adhesions have been found, either at necropsy or at operation, denoting that this illness must have been meningitis. At other times, though quite rarely, it is even possible to find at the base of the brain and elsewhere adhesions which could have been caused only by a pre-existing meningitis, although no illness may have been observed by the parents. These facts show the importance of a careful history of all patients suffering from intracranial pressure; they show that characteristic full-blown signs and symptoms of meningitis are not always present; and that the post-meningitic adhesions are not necessarily in proportion to the severity of the attack of meningitis. The situation, not the extent, of the post-meningitic adhesions determines the onset and severity of the resulting hydrocephalus. Communicating hydrocephalus can, of course, result from obstruction of the cisternæ by tumors of the pons and mid-brain and even by tumors situated in the middle cranial fossa; instances of these relationships have been reported in previous papers.[6, 10] The effects on the circulation of the cerebrospinal fluid are exactly the same whether the occlusion of the subarachnoid space is caused by tumor or by adhesions; but because of differences in treatment, the consideration of occlusions by neoplasms will not be considered here.

In seven out of ten patients with communicating hydrocephalus studied by cerebral pneumography, the obstruction has been located in the pontine or mesencephalic cisterna. In each of these, the column of air ended abruptly under the pons or mid-brain and no air reached the cerebral sulci; in each, the air passed freely into the lateral ventricles, demonstrating the free communication between the ventricles and the spinal subarachnoid space; in each, the cisterna magna was also seen, but its size varied greatly. In one instance it was scarcely visible, doubtless because adhesions between the cerebellum and the adjacent dura had obliterated this usually large chamber of fluid. In other instances the cisterna magna was greatly enlarged—even to the same degree as the fourth ventricle (Figs 9, 10). The pontine and mesencephalic cisternæ showed some variation in size.

Several times, when performing cerebellar operations on patients with hydrocephalus, I have been impressed with the fact that the cisterna magna, which usually covers nearly one-half of the posterior surface of both cerebellar lobes, was very small and at times were scarcely recognizable. Invariably in

[6] Dandy, W. E.: *Loc. cit.*

[10] Dandy, W. E.: Localization or Elimination of Cerebral Tumors by Ventriculography. Surg., Gynec. and Obst. April, 1920, p. 329.

these cases the cerebellum was tightly bound to the dura by adhesions. These operative findings, together with the necropsy observations, explain the cause of the pneumographic variation in the size of the cisternæ.

It has, doubtless, occurred to many, as to ourselves, to ask why hydrocephalus should be *internal* when the fluid can pass from the ventricles to the exterior. The fact that the cerebrospinal fluid forms in the ventricles and that the fluid is dammed back to its source only partially answers this question. The full answer is now clear. When an obstruction exists in the mesencephalic or pontine cisternæ, the extraventricular distribution of cerebrospinal fluid is restricted to the subarachnoid spaces in the posterior cranial fossa, and usually these spaces are reduced to less than normal size by the post-inflammatory process. The cisterna magna will be proportionately as large as the fourth ventricle when its enlargement is not precluded by adhesions; in other words the accumulation of cerebrospinal fluid and the dilatation of the fluid-containing spaces will occur up to the obstruction (the causative lesion) and the size of the various collections of fluid in these spaces will be dependent on the resistance offered.

Occlusion of All the Main Branches of the Cisterna Interpeduncularis and the Cisterna Chiasmatis

In two of our cases the occlusion was not in the cisternæ but in the large branches which radiate from the cisternæ interpeduncularis and chiasmatica and which carry the cerebrospinal fluid to all the surfaces of the cerebral hemispheres. Although the anatomical features of the two cases differed greatly, fundamentally they were similar in that the cisternæ were patent but all the branches were sealed. In each case the clinical diagnosis of communicating hydrocephalus was established by the phenolsulphonephthalein test. In each the site of the obstruction was determined by cerebral pneumography. In one case the findings were verified by necropsy and the intraspinal color test; and in the other by operation and the clinical tests. There was nothing unusual in the history of either case, the disease having been noticed soon after birth and having progressed with the usual rapidity until a tremendously large head had resulted. In one a partial fluid balance eventually had been established, as frequently happens, and at the age of four the fontanelles had slowly closed. The other child was only eight months old and the rate of growth of the head had not diminished.

After the injection of air into the younger child, the roentgenogram showed the air to have stopped in the cisterna immediately behind the sella turcica, *i. e.*, at the cisterna interpeduncularis. Only a small amount of air had been injected into the spine, and this passed freely into the lateral ventricles, but not a trace could be found in the cerebral sulci, which normally should fill with greater ease than the ventricles. The cisterna magna and the cisternæ under the brain-stem were small. At necropsy the spinal canal was injected with India ink. The distribution of this color was exactly that of the air as shown by the roentgenogram. The ink did not extend beyond the cisternæ although it reached the region of the optic chiasm. On the other hand, all the recesses of the cerebral ventricles, as well as the entire subarachnoid space surrounding the cerebellum, were filled with the black suspension, showing that the ink had had ample opportunity to reach the cerebral sulci, but had been prevented from doing so by an obstruction. No adhesions were evident during removal of the brain, nor could any be found later on careful inspection of the brain. In all of our other cases of communicating hydrocephalus, adhesions had been found at necropsy and one or two of the basal foramina (Luschka and Magendie) had been included in these adhesions and their closure had resulted. But in this case the foramina of Luschka and that of Magendie were open and larger than normal. In the accompanying photograph (Fig. 19), a wire has been inserted into the three foramina to show their position and condition. The patency or closure of each of these foramina can always be demonstrated easily and absolutely if the probe is passed from the fourth ventricle outward along the lateral recess. The personal equation cannot enter into the determination.

But in the absence of any demonstrable adhesions, and in the presence of an intact cisternal conduit, why do not the cerebral sulci fill? We know that they cannot fill, because both the air before death and the ink after death have been unable to reach them, and by the two tests, either of which should be absolute, an obstructing lesion has been located in identically the same position. There can be only one explanation for the failure of fluid to reach the cerebral sulci, namely, the absence of the main branches which carry the fluid from the cisternæ to the cerebral hemispheres. These branches may be absent either because they have been obliterated by adhesions following meningitis or they may have failed to develop. The absence of adhesion leads me to suspect the latter to be the cause, though the proof is lacking. Weed[3] has shown the cerebrospinal spaces to be a secondary splitting of the peri-encephalic mesenchyme and their development to follow closely upon the opening of the basal foramina, which result from a gradual thinning of the walls of the fourth ventricle; prior to this time the ventricular system is closed. At times these foramina fail to develop[11] and hydrocephalus results, and doubtless the same agenesis, perhaps easier to understand, may account for the failure either of the cisternæ or of its branches to develop.

It is worthy of note that in this case the cisterna is small, whereas it should be larger owing to the accumulated fluid up to the obstruction. The absence of this expected enlargement must indicate a rigid wall, which might be of inflammatory origin or it might be the congenital impediment which prevented the further development of the cisternæ in fœtal life.

In the second case the pathological features at first glance will appear to show little in common with the preceding case. The pathology was disclosed by operation and not by necropsy, but as there was ample opportunity to observe the entire surface of the brain, excepting most of that in the poste-

[11] Dandy, W. E.: The Diagnosis and Treatment of Hydrocephalus resulting from the closure of the foramina of Luschka and Magendie. Surg., Gynec. and Obst., February, 1921.

rior cranial fossa, a post-mortem examination would be of little additional value. Air was injected into a lateral ventricle and not into the spinal canal. As mentioned above, the phenolsulphonephthalein test showed a communicating hydrocephalus. At two operations both hemispheres were explored, and over neither was cerebrospinal fluid found in the cerebral sulci. A huge cyst filled the base of the cranial chamber and extending upward pushed the brain away from the floor of the skull. The cyst extended from one side of the skull to the other, and on each side it was continuous with the cisternæ chiasmatica and interpeduncularis. In fact, on each side it was a direct extension of these cisternæ. Moreover, when the cyst was opened (on either side) the brain-stem could be seen as far as the pons, owing to the tremendous size of the cisternæ under the mid-brain and pons; and doubtless the medullary cisterna was of corresponding size. The chemical analysis of this vast accumulation of fluid showed it to be the same as the cerebrospinal fluid in the lateral ventricles. Furthermore, the phenolsulphonephthalein test demonstrated communication between the ventricles and these extra-cerebral cysts, but only after half an hour. In other words, the communication was by a devious path and was not direct. The absence of air in the cysts after ventricular injection also eliminates any direct communication, and finally, at operation there was seen to be no direct communication.[12]

We are dealing, therefore, with a case of communicating hydrocephalus in which the obstruction is at the branches which pass from the cisterna interpeduncularis and the c. chiasmatis to the surfaces of the cerebral hemispheres (there being no fluid in the cerebral sulci). The walls of the cisternæ, gradually yielding to the pressure of the accumulating fluid, allow the formation of the huge cysts instead of the usually restricted cisterna under the brain. No adhesions of note were found at either operation, so that the assumption of a meningitis would be without a history of this affection and without any anatomical evidence of its existence. The most plausible explanation of the cause of this condition is the congenital failure of the large branches of the cisternæ to develop. In the preceding case it is possible that the cisternæ may have been obliterated rather than that the branches have failed to develop; in this case the cisternæ are well open, in fact they are greatly distended; under the mid-brain the cisterna is as large as one's index finger.

An external hydrocephalus differs from the above picture only in that the fluid is distributed over the hemispheres instead of being confined to a localized cyst of more restricted size. In fact, in this case the hydrocephalus was transformed into an external hydrocephalus merely by opening the cyst, but the fluid, passing over the arachnoid membrane instead of under it, poured into the subdural space instead of the subarachnoid space, where there was only a slight absorption. The pathology of this case really forms a connecting link which explains the relationship between external hydrocephalus and internal hydrocephalus; this relationship and the general subject of external hydrocephalus will be considered in detail in a forthcoming publication.

Obstruction of Some But Not All of the Main Branches of the Cisternæ

In the accompanying ventriculogram (Fig. 14) evidence of a very early hydrocephalus will be seen. It developed in a three-year-old child under observation in the service of Professor Howland. She was first treated for a typical illness of acute meningococcus meningitis, from which there was an apparent recovery, though very shortly lethargy and vomiting ensued. Six weeks after the onset of the attack of acute meningitis and two weeks after apparent recovery,[13] hydrocephalus was first suspected. At this time, the cell count in the cerebrospinal fluid was ten. The sutures of the skull were separated; a suggestive cracked-pot sound was obtained. There was no choked disc and no other sign of intracranial pressure. Without the ventriculogram the diagnosis of hydrocephalus could never have been substantiated. With the absolute verification it is probably the earliest recorded case of hydrocephalus. The ventriculogram was different from that of any previous case which has come under my observation. Air passed from the ventricles and finally reached the cerebral sulci, but only in a very restricted segment over the frontal lobe. Such a finding might have been assumed to be due to an imperfect injection of air, but one month later another ventriculogram was made and precisely the same segment of subarachnoid space and exactly the same sulci were injected. In this interval of 30 days between the two ventriculograms, the lateral ventricle had increased in size (compare Figs. 14 and 15), though the rate of growth was considerably less than in the usual development of hydrocephalus. The phenolsulphonephthalein output from the spinal canal rose from 13 per cent to 22 per cent; the latter percentage was found at the time the first ventriculogram was made; unfortunately, no test was made when the second ventriculogram was obtained. It is evident, from the phenolsulphonephthalein test, that a partial compensation has occurred, for, with 22 per cent absorption (normal 35-50 per cent), the hydrocephalus could not be full-blown. The ventriculogram was also interpreted to mean that the filling of part of the subarachnoid spaces denoted that a greater amount of cerebrospinal fluid (12 per cent more by the phenolsulphonephthalein test) than in the usual quantitative absorption in communicating hydrocephalus with an obstruction in the cisterna was being absorbed in this restricted area and that a partial compensation had developed, as it should have done.

[12] A description of the remarkable anatomical changes in this case would only add confusion if presented here. They are therefore omitted and will appear in a subsequent publication dealing with other phases of hydrocephalus. My purpose here is to correlate all the anatomical variations of communicating hydrocephalus into a single disease with a fundamentally similar pathology and etiology.

[13] A pneumococcus panophthalmitis also developed, necessitating removal of the eye, from which the meningococcus was grown in pure culture.

The branches of the cisternæ can be traced directly from the cisterna chiasmatis to the cerebral sulci of the frontal region. Numerous perpendicular air shadows are clearly shown just above the cisterna interpeduncularis and rising vertically from it but ending blindly. These shadows, I believe, represent the branches of the cisternæ which are obstructed and which should supply the remainder of the cerebral hemispheres with fluid. The air extends in these branches up to the point of the obstruction in each individual branch (Fig. 14).

This patient has been seen at intervals for the past two years and has recovered completely. We have proof from the pneumographic records not only of the existence of a hydrocephalus, which could not have been diagnosed otherwise, of its unusual rate of development, and of its spontaneous cure, but, more important, we have the findings in the transition stages and, we think, the reason for the compensation and eventual cessation of development of the hydrocephalus. Whether at one time *all* the cisternal branches were occluded and *some* subsequently opened, producing a partially compensating hydrocephalus; whether additional spaces were reestablished after our studies were made and permitted the condition to change from a partially compensating hydrocephalus to a complete cure, we have not the pneumographic evidence to prove or disprove.

I do not believe that it is possible for a hydrocephalus to occur, if all or even many of the cerebral sulci can be shown to fill with air or if the phenolsulphonephthalein output after a spinal injection measures 35 per cent in two hours. From a large series of cases of hydrocephalus, there has been no exception to disprove this statement. We are, however, not yet sufficiently familiar with the roentgenographic pictures of the cerebral sulci to make many positive claims as to prognosis in these unusual types of hydrocephalus.

SUMMARY AND CONCLUSIONS

(1) The cerebrospinal fluid circulates in a closed vascular system. This is just as well defined as the vascular system for blood, lymph, bile or urine.

(2) The ventricular system, in which fluid is produced but not absorbed, is lined with a high cubical and columnar epithelium; the subarachnoid space, in which the cerebrospinal fluid is absorbed, is lined with low mesothelial cells. Nearly all the cerebrospinal fluid is absorbed in the cerebral sulci.

(3) Collateral circulation is almost precluded either in the ventricles or in the cisternæ. An obstruction in these spaces, therefore, results in a hydrocephalus, just as closure of a ureter results in a hydronephrosis. If the obstruction is situated in any part of the ventricles (usually the aqueduct of Sylvius or the foramina of Luschka and Magendie) the hydrocephalus is of the obstructive type; if it is situated in the cisternæ (or in the main branches of the cisternæ) it is of the communicating type.

(4) That the cause of communicating hydrocephalus (the remnant of so-called idiopathic hydrocephalus) is an obstruction in the cisternæ is conclusively demonstrated in three ways. (a) Experimentally communicating hydrocephalus can be produced by blocking the mesencephalic cisterna. (b) The obstruction can be graphically demonstrated in the experimental animal or at necropsy in the human by injecting a suspension of India ink into the spinal canal; the color stops abruptly at the obstruction. (c) In all living patients the obstruction can be clearly shown by cerebral pneumography after air has been injected into the spinal canal; the air also stops at the obstruction, and can be sharply outlined in the roentgenogram.

(5) The obstruction in the subarachnoid space is most frequently located in the mesencephalic or pontine cisterna.

(6) However, the obstruction need not necessarily be in the cisternæ; it may be in the large branches which carry the fluid from the cisternæ chiasmatica and interpeduncularis to the cerebral sulci. Any number of these branches may be occluded. If all the main branches are obstructed, the hydrocephalus will be the same as if the occlusion were in the cisterna. If some of the branches remain unobstructed, the degree of hydrocephalus will be modified proportionately; a complete cure may even result because of the absorption which takes place in the remaining patent areas of the subarachnoid space.

(7) Adhesions, which follow meningitis and occlude the cisternæ, cause the vast majority of cases of communicating hydrocephalus. They also cause many cases of obstructive hydrocephalus, by blocking the foramina of Luschka and Magendie. Adhesions give infallible proof of a preexisting meningitis. A history of meningitis may be easy, difficult or impossible to obtain. The post-meningitic occlusions have no relation to the severity of the attack and the number of adhesions but rather to the location of the adhesions.

(8) In two cases the hydrocephalus appeared to be due to a congenital failure of the cisternæ or of its branches to develop. Tumors in the pons, medulla, or mid-brain also produce partial or complete obstruction of the subarachnoid space and therefore cause communicating hydrocephalus.

(9) Pneumographic records are shown demonstrating the existence of a very early stage of communicating hydrocephalus, the cause of the hydrocephalus, the reason for its unusually tardy development, and for its spontaneous arrest.

DESCRIPTION OF PLATES

FIG. 1.—The mesencephalic cisterna is completely blocked in a dog by surrounding the mid-brain with a piece of gauze saturated with iodine. The lower figure shows a section of the mid-brain with the gauze band in place. The band is between the margins of the incisura tentorii and the surface of the mid-brain. Communicating hydrocephalus follows this experimental procedure; the reasons for the hydrocephalus will be seen in Figs. 3 and 4.

FIG. 2.—Section of a dog's brain to show the grade of hydrocephalus which resulted from the perimesencephalic band of adhesions (Fig. 1). On the right is a section of a normal brain as a control. The hydrocephalus is of three months' development.

FIG. 3.—Drawings by Max Brödel to give a graphic demonstration of the reason for the development of communicating hydrocephalus after the formation of the perimesencephalic adhesions. India ink had been substituted for cerebrospinal fluid in the spinal canal two hours before the animal was sacrificed. On the right is a control

animal in which the same quantity of ink was injected at a similar time before death. In each case the ink has had the distribution which the cerebrospinal fluid would have. In the normal (right), the ink has thoroughly and evenly covered every part of the brain's surface because all of the cerebrospinal spaces receive the cerebrospinal fluid. In the experimental animal the ink has stopped sharply at the perimesencephalic band. None of the ink has been able to reach the cerebral hemispheres, although the cerebellum has been as well covered as the normal. In Fig. 2 it will be seen that in the normal brain the ink has not entered the lateral ventricles, whereas in the experimental animal the ventricles have filled with ink. Since the ventricles fill with ink, there can be no question that the injection is inadequate, for the ventricles are farther forward than the obstructing band.

Fig. 4.—Dorsal view of the same brains (as Figs. 2, 3) to show the distribution of the ink on this surface. It will be seen that the ink has not extended beyond the tentorium (owing to the perimesencephalic band), whereas in the normal the entire brain is covered. Hydrocephalus results from this band because the trunk of the subarachnoid tree is occluded and cerebrospinal fluid cannot reach the spaces over the cerebral hemispheres where most of the cerebrospinal fluid absorbs.

Fig. 5.—Drawing by M. Brödel to show the general plan of the vascular system for cerebrospinal fluid. Fluid forms in the cerebral ventricles and is absorbed in the subarachnoid space. The paired foramina of Luschka and the median foramen of Magendie are the only openings by which the ventricular fluid can leave the ventricles and reach the subarachnoid space. Obstructions at these openings, the aqueduct of Sylvius or at the foramen of Monro produce hydrocephalus involving the ventricles anterior to the obstruction. Obstructions in the subarachnoid space are just as effective in producing hydrocephalus. The sites of these obstructions and their effects will be shown in the succeeding diagrams.

Fig. 6.—Pneumogram (intraventricular injection of air) to show normal ventricle and normal subarachnoid spaces. Note that the cerebral sulci (the wavy lines) are filled over the entire surface of the brain. The cisternæ interpeduncularis and chiasmatica are shown as the distributing center from which all the cerebral sulci receive their fluid.

Fig. 7.—Diagram to show the disturbance in the circulation of cerebrospinal fluid following an obstruction at the mesencephalic or pontine cisterna. The black area represents the absorbing spaces, which cannot be reached by the cerebrospinal fluid owing to the obstruction. Hydrocephalus results because this vast area (where three-quarters to four-fifths of the cerebrospinal fluid is normally absorbed) can no longer perform its function. The mesencephalic and pontine cisternæ are the usual sites for post-meningitic obstructions in communicating hydrocephalus. A pneumogram of this type of obstruction is shown in Fig. 9. Fig. 8 shows a patient with this disease and having an occlusion at this point; Fig. 10 shows the ventricular system of this patient filled with air. Figs. 9 and 10 show this obstruction demonstrated clinically by the intraspinous and intraventricular methods, respectively. Figs. 11 and 12 show the pathology of this type of hydrocephalus.

Fig. 8.—Photograph of a patient with communicating hydrocephalus following meningitis. The marked retraction of the head and neck has persisted long after the acute illness has subsided. The ventriculogram of this patient is shown in Fig. 10.

Fig. 9.—Cerebral pneumogram (retouched) of a case of communicating hydrocephalus; air has been injected into the spinal canal. The obstruction is at the mesencephalic cisterna (*cf.* arrow). No air has reached the cerebral sulci but the lateral ventricle has been partially filled by the retrograde flow of air through the dilated foramina of Luschka and Magendie. Compare this *intra vitam* demonstration of the causative lesion with that shown *post mortem* by the injection of ink (Figs. 17 and 18) or with the example of experimental hydrocephalus (Figs. 2, 3, and 4). A = cisterna pontis. B = cerebellar subarachnoid space. C = cisterna magna. V = lateral ventricle.

Fig. 10.—Untouched reproduction of a ventriculogram from a case of communicating hydrocephalus (shown in Fig. 8); 800 c. c. of fluid were aspirated and an equal quantity of air substituted. The entire cerebrospinal vascular system is shown in the pneumogram up to the point of obstruction, which is also sharply defined. The tremendous lateral ventricles have lost all semblance of their former shape and practically fill the huge cranial chamber. The third ventricle, the aqueduct of Sylvius, and the fourth ventricle are outlined sharply. The foramen of Magendie can be seen; the large cisterna magna fills much of the posterior cranial fossa. The obstruction which is causing the hydrocephalus is at the anterior terminus of the shadow of the cisterna magna. The obstruction, therefore, is in the cisterna pontis; no air has reached the cerebral sulci. The hydrocephalus in this case followed an attack of epidemic meningitis. V = lateral ventricle. III = third ventricle. IV = fourth ventricle. P = suprapineal recess of third ventricle. $A.S.$ = aqueduct of Sylvius. M = foramen of Magendie. C = cisterna magna. X = obstruction in pontine cisterna. This obstruction causes the hydrocephalus.

Fig. 11.—Inferior surface of the brain of a case of communicating hydrocephalus; the mid-brain has been divided transversely and the cerebellum and brain stem removed. Note the large aqueduct of Sylvius B and the large tumor-like dilated third ventricle A which compresses the optic nerves and destroys the sella tursica just as a hypophyseal tumor would do. Such a protrusion of the third ventricle is not always present in hydrocephalus.

Fig. 12.—Inferior surface of the brain stem and cerebellum of the above case (Fig. 11). The foramen of Magendie is entirely closed by adhesions. The right foramen of Luschka is patent (reader's left) as indicated by the probe L which easily passes through it from the fourth ventricle. The left foramen of Luschka (reader's right) is sealed and protrudes as a bulging cyst. The probe which is introduced into the lateral recess of the fourth ventricle of this side meets this obstructing membrane. The dilated cystic pouch (foramen of Luschka) is surrounded by a series of arrows in order to identify it.

Fig. 13.—Diagram showing the effect on the cerebrospinal fluid circulation when some, but not all, of the branches from the cisternæ interpeduncularis and chiasmatica are occluded. The black area represents the subarachnoid space which does not receive cerebrospinal fluid. In the clear zone the circulation is intact. Figs. 14 and 15 are pneumographic records of such a type.

Fig. 14.—Photograph of roentgenogram of head after the injection of air into ventricle (not retouched). This patient had an early hydrocephalus following acute cerebrospinal meningitis. Only a small number of the cerebral sulci contain air (bracket between x and x). The obstruction which caused the hydrocephalus was not in the cisternæ but in the large branches which carry the cerebrospinal fluid from the cisternæ to the sulci. The arrow points to the cisternæ and the dilated obstructed branches; C = cisterna magna. The shadow of the mesencephalic and pontine cisternæ can be followed through the dense petrous bone. The partial filling of the cerebral sulci (x to x) explains the slow development of the hydrocephalus and its subsequent spontaneous cure. Fig. 15 is a ventriculogram of the same case 30 days later.

Fig. 15.—Pneumogram of above case (Fig. 14) 30 days later. The increase in size of the ventricle is clear and easily measurable, but it is of a moderate grade. Exactly the same sulci are injected and the same dilated cisternæ interpeduncularis and chiasmatica and the same dilated obstructed branches are shown.

Fig. 16.—Diagram showing the effect upon the cerebral subarachnoid space when all the main branches, which carry the fluid from the cisternæ interpeduncularis and chiasmatica, are occluded. Exactly the same absorbing area is eliminated and the hydrocephalus which results is identically the same. In our cases of this type absence of these branches was regarded as probably due to a congenitally defective development of these spaces. Fig. 20 is a ventriculogram of this

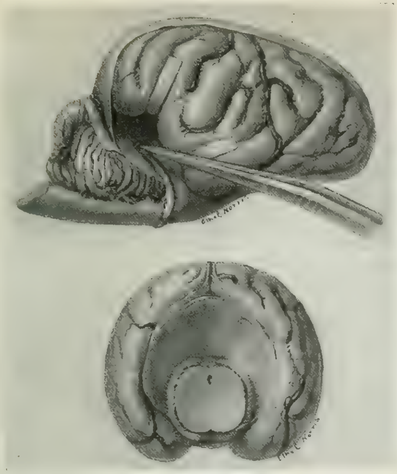


FIG. 1.

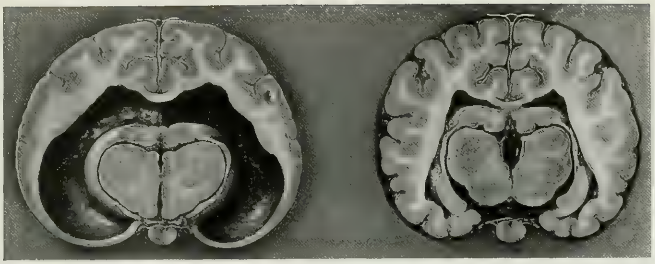


FIG. 2.

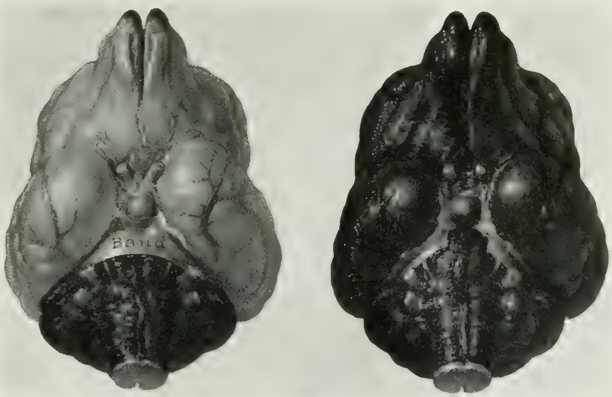


FIG. 3.

FIG. 4.

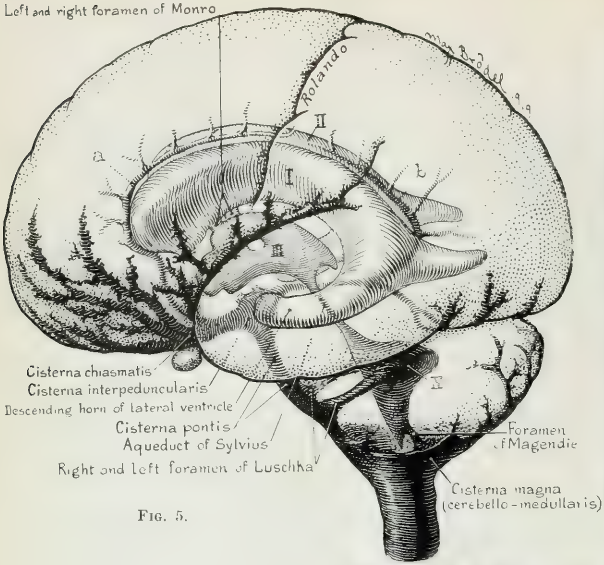


FIG. 5.

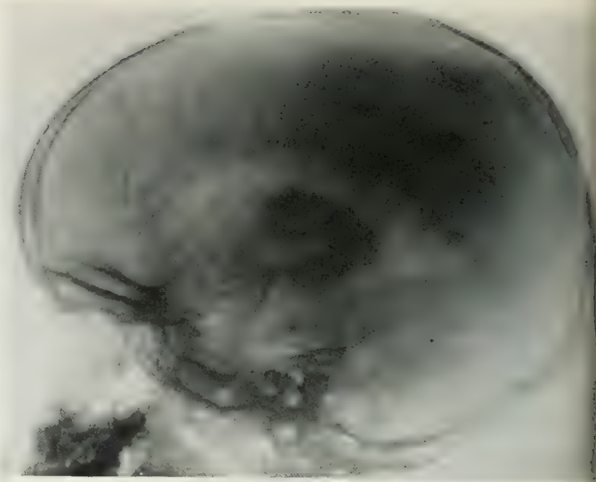
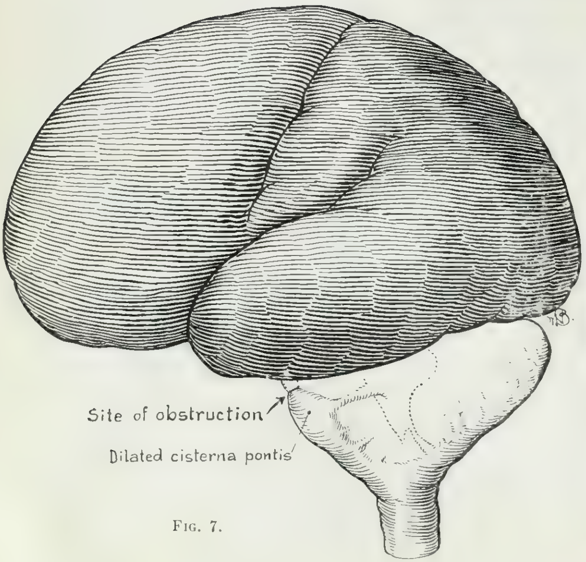


FIG. 6.

FIG. 8.



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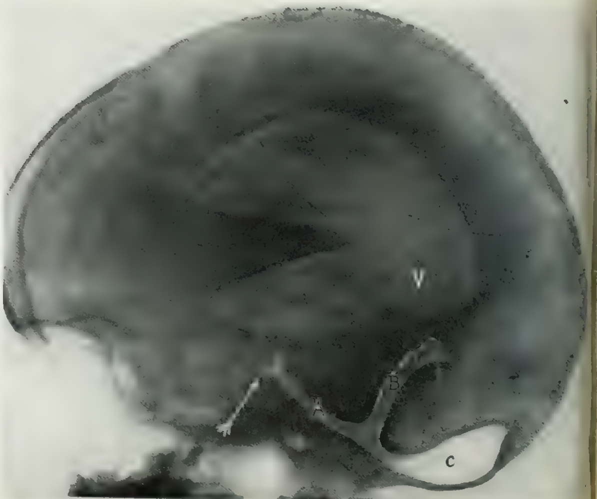

FIG. 9.

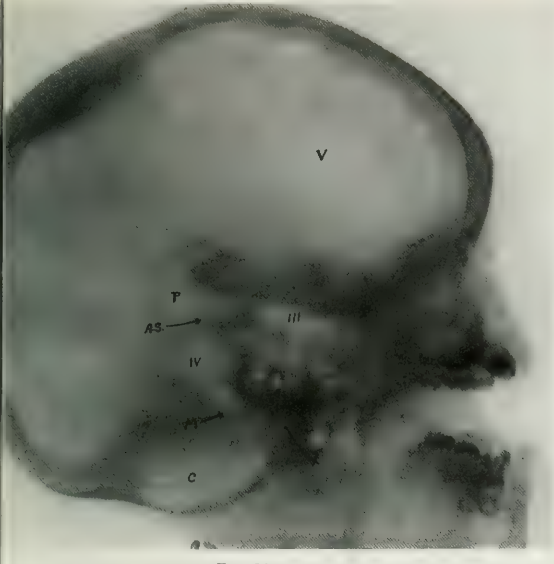

FIG. 10.

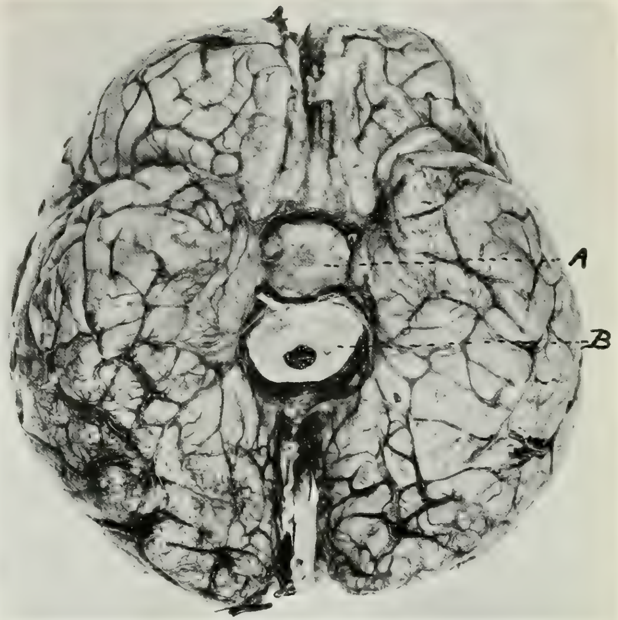

FIG. 11.

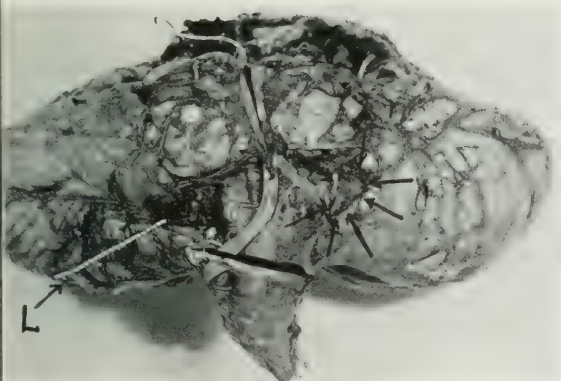

FIG. 12.

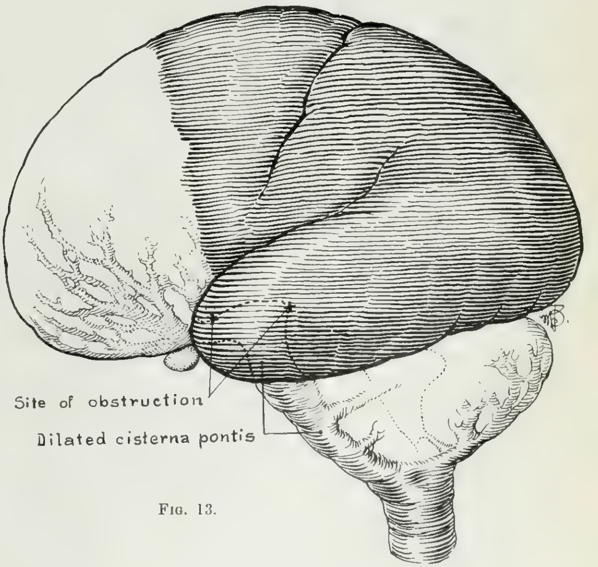

FIG. 13.

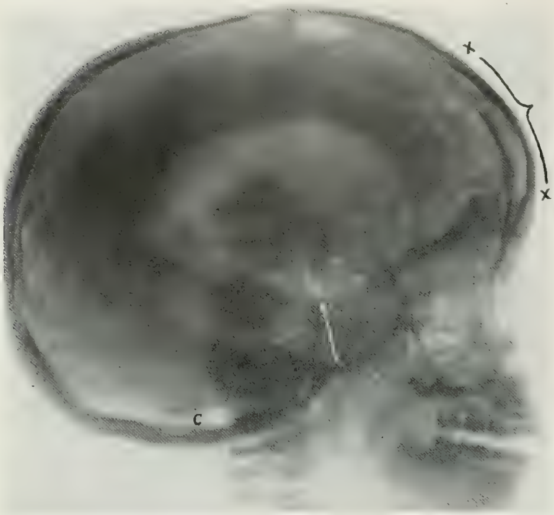

Fig. 14.

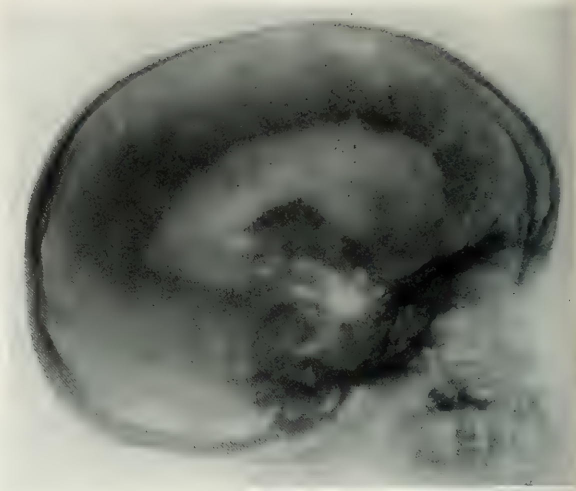

Fig. 15.

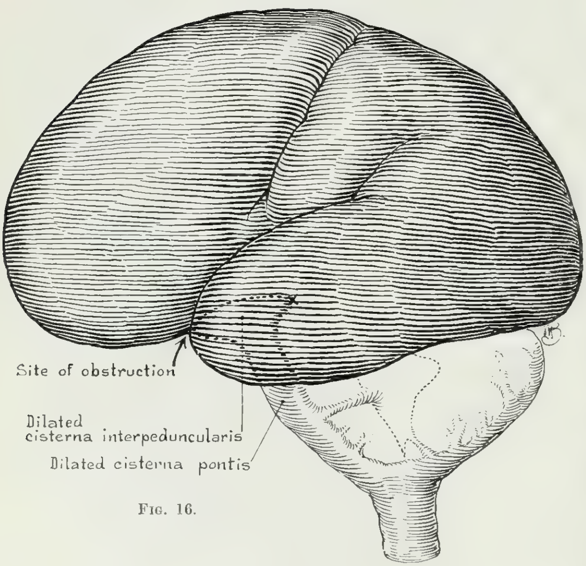

Fig. 16.

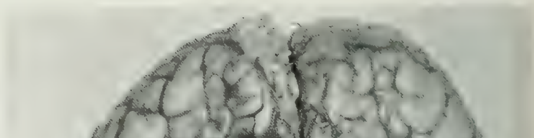

Fig. 17.


Fig. 18.


Fig. 19.


Fig. 20.


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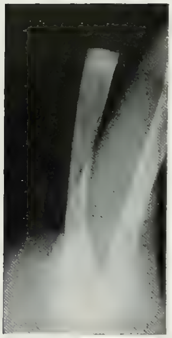
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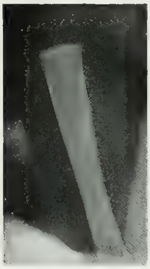
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[In the description of figures which have been made from the fetal bones examined, the number CC indicates the number of the specimen in the Embryological Collection of the Carnegie Institute.]

1. Fetal osteochondritis luetica. Roentgenogram from the distal end of the femur. Fetus CC No. 2333. This picture shows excessive calcification.

2. Hand and forearm of human fetus (CC No. 2300) to show extreme excessive calcification of the provisional area with irregular prolongation of the provisional calcified zone into the area of proliferative cartilage. Note the presence of the same lesions in the metacarpals and phalanges.

3. Radius and ulna from human fetus (CC No. 2186) showing beginning resorption of the area of intense calcification at the epiphyseo-diaphyseal junction. Resorption shown by areas of decreased density of shadow, each resorptive area surrounding a small nucleus of persistent trabecular tissue

4. X ray picture of syphilitic osteochondritis of the bones of the hand and forearm of a human fetus (CC No. 2044) showing a zone of rarefaction between two lines of abnormal calcification Note the lesion in the phalanges and metacarpals.

5. Human fetus (CC No. 2350). Luetic osteochondritis of the distal end of the femur. This bone shows an abnormally heavy calcification of the provisional area of calcification separated proximalward by a narrow line of rarefaction from the broad band of dense shadow cast by fine closely set trabeculæ at the epiphyseal end of the shaft.

6. Third stage (Wegner) of luetic osteochondritis of the distal end of the femur and proximal ends of tibia and fibula. Human fetus CC No. 2643. This is the picture which immediately precedes epiphyseal separation. A wide band of syphilitic tissue separates the irregular broad spotty zone of provisional calcification from the distal end of the diaphysis.

7. Syphilitic periostitis of both bones of the forearm of fetus CC No. 2597. Note the longitudinal striation of the thick periosteal shadow which is nearly in contact with the shafts of the bone.

8. Syphilis of the femur. Human fetus CC No. 2513. Showing separation of the cortex from the spongiosa.

9. Distal end of radius and ulna of Case I. This plate shows intense calcification of the provisional zone with resorption areas on the marrow side of the epiphyseal line. Both bones show syphilitic periostitis and there is separation of the cortex from the spongiosa in the ulna.

10. Radiogram of the distal ends of the radius and ulna of Case II. This shows a syphilitic osteochondritis with marked areas of resorption and a mild grade of periostitis.

11. Roentgenogram of the proximal ends of the tibia and fibula of Case III, showing syphilitic osteochondritis with over-calcification of the provisional calcified zone with a Trümmer-zone-like area behind it and a mild grade of periostitis of the tibia.

type of obstruction. Figs. 17 and 18 show the obstruction as defined by an intraspinous injection of ink.

Fig. 17.—Base of brain of a case of communicating hydrocephalus. Ink has been injected into the spinal canal before necropsy. The black coloring extends along the cisternæ to the optic chiasm. It has covered the cerebellum but none of the cerebral hemispheres. (The black shadow over part of the left temporal lobe (reader's right) is due to a post-mortem hæmatoma and not to the ink.) Compare this post-mortem demonstration of the obstruction with a pneumographic demonstration (Fig. 9) during life and with the experimental lesion (Figs. 1, 2, 3, and 4).

Fig. 18—Sagittal view of the above brain (Fig. 17). The ink is seen deeply staining the walls of the third and fourth ventricles and the aqueduct of Sylvius, although none of the color reached the surface of the cerebral hemispheres. The aqueduct of Sylvius and the foramen of Magendie are patent and enlarged, thus demonstrating that the hydrocephalus is of the communicating type. Compare the intraventricular distribution of ink in the experimental animal (Fig. 2).

Fig. 19.—Cerebellum and brain stem of the above case (Figs. 17 and 18). The three wires are intended to show that the three foramina (Luschka and Magendie) are open. On the right side the wire had slipped out and was replaced by the photographer, who thought it had been properly reinserted. The foramen is seen *under* the wire, the border of the foramen being indented by its pressure; the actual foramen of Luschka can be seen in the circle of arrows; the flocculus emerges from the foramen of Luschka on each side.

Fig. 20.—Ventriculogram of a case of communicating hydrocephalus due to occlusion of the main branches of the cisternæ interpeduncularis and chiasmatica. The larger two-finger-like process projecting into the ventricle is a huge cyst, which is a direct outgrowth of the cisternæ interpeduncularis and chiasmatica. The elucidation of the findings was possible by a thorough inspection of the base of the brain at operation. Owing to pressure on the aqueduct of Sylvius the circulation of air from the lateral ventricles was restricted; therefore, the cyst, the fourth ventricle and the cisternæ did not immediately fill with air following the intraventricular injection.

X-RAY PICTURES OF THE BONES IN THE DIAGNOSIS OF SYPHILIS IN THE FETUS AND IN YOUNG INFANTS

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and

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For some time past it has been quite generally recognized that but little dependence could be placed on the Wassermann test during the early weeks of life as an aid to the diagnosis of syphilis. A positive Wassermann is still regarded as nearly specific but a negative serum test no longer is allowed to free the newly born child or young infant from suspicion of luetic infection. Certainly this is true until the end of the second month of life is reached and there is a growing tendency to extend the period of capricious serum reaction to the end of the fourth month. Indeed there is so little expectation of obtaining a positive complement fixation reaction from the blood of newly born children that in the obstetrical service of at least one of our large teaching hospitals [1] the routine syphilological examination of blood from the cord of the newly born babies is regarded as not repaying the trouble and expenditure involved in carrying out the technique.

The diagnosis of lues in the newly born or very young child is not by any means always easy, since clinical symptoms of the disease may be, and in fact are usually, entirely absent from the child until several weeks after birth. Moreover, a mother without a single clinical manifestation or any serological indication of spirochætal infection may give birth to a luetic infant.

Under such conditions, when any addition to the diagnostic armamentarium against the disease in young children might be welcomed, it seems rather surprising that so little attention has been paid to the routine use of a method which has the advantage of rapidity and ease of application—the examination of the bones by means of the X-ray.

It has long been a matter of common knowledge that the skeleton is among the tissues most frequently affected by the luetic process during intra-uterine life and as early as 1870 Wegner described three stages of osteochondritis syphilitica. Moreover, the alterations in the affected bones are easy of recognition in the X-ray picture and when present are pathognomonic.

Every syphilitic infant does not show bone lesions on X-ray examination, but cases are not infrequent among young children in which roentgenography discloses evidences of lues when no clinical or serological data are available for diagnosticating the presence of the infection. Our experience has led us to believe that routine examination of the osseous system in newly born children will yield valuable diagnostic data and insure recognition of the presence of a hereditary infection in a certain number of children who otherwise might go on unaided for several months before some clinical symptom or the accidental discovery of a positive serum reaction would secure for them the much needed therapy.

We have had the opportunity of examining some 300 white fetuses ranging in age from the sixth month of intra-uterine life to nearly term with the object of securing as many variations as possible of the normal and pathological skeletal X-ray pictures. The fetuses were of white parentage and were listed as normal in the catalogue of the Carnegie Institute of Embry-

ology, from which collection they were taken.* Incidentally, the examination of the plates taken of these specimens furnished a striking demonstration of the terrible toll claimed by syphilis during intra-uterine life.

Out of the first 100 plates studied, representing the same number of white male fetuses, 15 showed advanced luetic osteochondritis, 10 had signs of less marked syphilitic involvement and 21 showed one or more bones which presented slight variations from the normal picture and were noted as suspicious. In other words, the skeletons of 25 per cent had marked signs of lues and 46 out of the first 100 bodies examined had well-marked or suspicious lesions.

The syphilitic lesions seen in the bones of children are roughly of two types, those seen in newly born and young infants and those which characterize syphilis of the osseous system of older children. We shall not attempt to discuss here those of the latter variety and, since those which it is necessary to recognize in infants and newly born children are essentially of the fetal type, a detailed knowledge of the lesions encountered in the fetus is most important in the routine use of the X-ray picture as a diagnostic method.

If one examines an X-ray picture of a normal fetus or newly born child, it will be seen at once that while the shadow cast by the diaphysis is sharply outlined, the epiphyseal cartilage is invisible, since its density is approximately equal to that of the surrounding soft parts. The cortex of the shaft, which is usually of unequal width according to the curvature of the bone, is thickest at the plane of entrance of the nutrient canal and from this point it tapers in either direction to hairline thinness at the epiphyseo-diaphyseal junction. In the central canal of the bone the marrow space is very small and one sees the spongiosa made up of sharply cut, fine, regularly ordered trabeculæ arranged in the form of a framework for the homogeneous marrow spaces about the circulatory system of the bone. The nutrient canal, through which the blood vessels enter the bone, is easily made out and can be seen to divide into two branches just beneath the cortex. The bony trabeculæ become finer and finer as the epiphyseo-diaphyseal junction is approached and end abruptly in a more or less curved, or straight, but always clear-cut line which is so sharply drawn as to appear to be ruled. They may terminate in a fine granular line running at right angles to their long axes (the calcified intracellular substance of the epiphyseal cartilage) but usually the individual trabecula may be distinguished throughout its entire length.

Syphilitic bones throw quite a different and characteristic shadow on the photographic plate after exposure to the X-ray. Usually all the bones are not affected to the same extent and some of them may have apparently escaped entirely. Those which are most often or most severely affected are in order of frequency the lower end of the femur, the distal and proximal ends of the tibia, the distal ends of the radius and ulna, the extremities of the metacarpals, the proximal ends of the phalanges, and the proximal ends of the ulna and radius. No bones are exempt from the syphilitic changes; even the bodies and processes of the vertebræ, the ribs and the bones of the skull do not escape.

The shadows resulting from syphilitic lesions in early life are due to vagaries in the calcification of the provisional cartilage and to the abnormal arrangement and distribution of osseous tissue. The syphilitic changes in the bones of the fetus, unless they are very severe and of long standing and the fetus is close to term, do not involve the periosteum to any demonstrable extent but are confined to the epiphyseo-diaphyseal region. At any rate in the fetal type of reaction the periosteal lesion is secondary in importance to the endochondral defect. After birth the periosteal reaction begins, possibly because of the increased demands made on this tissue by the increased muscular activity, and in young infants this may be the most marked skeletal lesion.

The beginning of the process as shown by the X-ray picture is an intensification of the shadow cast by the bone at the epiphyseal line. This line becomes much broader and more homogeneous and seems to form a cap on the ends of the trabeculæ of the spongiosa (Fig. 1). This is significant of the beginning of abnormally heavy calcification of the provisional calcified zone. It must be remembered that while the provisional zone of calcification in the cartilage of the normal embryonic bone is, relatively speaking, very narrow, in many cases only one or two cells deep, in the syphilitic bone the calcified cartilage may show on section a width of from 0.5 mm. to 1.5 mm.

In other bones in which the osteochondritis is further advanced, it can be seen that on the marrow side of the intensified shadow of the provisional zone there is a band-like area where the shadow is less intense than in the rest of the bone (Fig. 3), giving an appearance of diminished density to the region of the epiphyseal line.

Bones may also be seen in which the dense shadow at the epiphyseal end of the bone is broken by the presence of one or more small areas of rarefaction so as to give an appearance of irregular density to the end of the bone (Fig. 3).

At other times the bone appears to end in a double line, so that two lines of heavily calcified tissue are seen, separated each from one another by a zone in which lime salts are less heavily deposited. This zone is a region which histological preparations show to contain a great deal of delicate granulation tissue. This picture becomes more and more intensified as growth goes on. The areas of dense shadow and the fine clearer band between them grow wider and the surfaces bounding them become more and more irregular and jagged till the end of the bone has an irregular, ragged appearance (Figs. 4, 5, 6). During the course of the disease the calcification of the infected areas is not only abnormally heavy but also most irregular, so that the epiphyseal border of the shadow cast by

* We have here to express our gratitude to Dr. Geo. Streeter, director of the Laboratory of Embryology of the Carnegie Institute, for permission to use this material, and to Miss M. S. Smith of the X-ray Department of The Johns Hopkins Hospital for the time and care spent in making excellent X-ray plates from most difficult material.

the bone has a notched, saw-toothed or serrated appearance (Fig. 2).

Practically all the bones are involved by the luetic process and it has been shown by Alexander,[2] who has studied the roentgenography of osteal syphilis with great care, that luetic changes can be demonstrated in the excised ribs, in the scapula, vertebral bodies and processes and in the bones of the skull base and pelvis. About the various centers of ossification in the different bones of the body the syphilitic picture is beautifully drawn. The nucleus becomes surrounded by a very wide zone of provisional calcification and eventually becomes doubly contoured by the development of a pericentral ring of tissue which casts but little shadow.

The phalanges, metacarpals and metatarsals give characteristic pictures of luetic osteochondritis and are affected by the spirochæte with surprising regularity and to a very marked degree (Figs. 2, 3, 4). The metacarpal and metatarsal bones show the lesion at either end, but the phalanges, because of the manner in which they are developed, are affected only at the proximal extremities.

Periostitis when it occurs near term in the severe cases of lues may be present throughout the length of the bone or only at the extremities. It is shown in X-ray plates by a more or less wide, almost homogeneous, shadow or with longitudinal striations separated from the external surface of the cortex by a narrow clear area which bounds the bone (Fig. 7).

One other feature of these pictures appears worth noting. It may be seen that in many luetic bones the cortex is separated from the spongiosa by a very narrow clear zone which gives the cone of spongy bone the appearance of being suspended unattached within the cortical cavity (Fig. 8). In the X-ray picture the trabeculæ of the syphilitic bone appear to be finer than those of the normal bone.

With these facts in mind it will be seen that osteal syphilis may be easy of recognition even in the fetus and that X-ray studies, if employed as a routine, may be at times a valuable aid in the early diagnosis of hereditary lues.

The following cases serve to illustrate the value of the procedure:

Case I.—M. D., colored, female.

First seen in the out-patient department of the Harriet Lane Home on April 19, 1918, at the age of 5 weeks with the complaint that she did not use the right arm well. There were no signs of syphilitic infection and nothing in the history to suggest hereditary lues. The Wassermann test was negative, but the X-ray picture (Fig. 9) of the bones of the forearm showed a slight degree of periostitis and a well marked syphilitic osteochondritis of the radius and ulna. *Diagnosis.*—Syphilis, hereditary, early (epiphysitis).

Case II.—H. R., white female infant, aged 6 weeks.

Came to the out-patient service at the Harriet Lane Home September 30, 1920, with a slightly swollen wrist-joint. There was no suggestion of any syphilitic history and examination for signs of the disease was without result. Repeated Wassermann tests were negative, but the X-ray plate of the swollen wrist (Fig. 10) showed a syphilitic osteochondritis with a very intense grade of resorption behind the epiphysis. This child was given antisyphilitic medication and the family kept under observation. It was possible to obtain blood from both the mother and father of the patient and a Wassermann reaction was done in duplicate in two different laboratories on both parents, once with serum from the father and twice with the mother's blood. Both laboratories returned a negative report on the paternal serum. The report on the mother's serum was twice returned by one laboratory as positive and twice negative by the other. The child is still under treatment with diarsenol and mercury and has shown marked improvement.*

Case III.—K. P., white female child under observation in the out-patient department of the Harriet Lane Home since June 10, 1920, aged 2 months, with a diagnosis of hypertrophic stenosis of the pylorus. Physical examination was negative except for a somewhat enlarged spleen and a slightly swollen ankle. The child's Wassermann reaction was negative three times, on the 1st and 11th of November, and on the 2d of December, 1920. The mother's Wassermann was negative on the 11th of November. The X-ray picture of the swollen leg (Fig. 11) showed syphilitic osteochondritis of both proximal and distal ends of the tibia and fibula. Since November 2d the child has improved under treatment with mercury and diarsenol.

Two other conditions which are encountered in children may give X-ray pictures which closely resemble, and in some cases are identical with, the pictures described above. Scurvy and rickets, when the latter disease is healing under the influence of cod-liver oil therapy, may be difficult or impossible to differentiate by roentgenographic means from osteal syphilis of the fetal type. Fortunately, however, in the early weeks of life neither of these conditions need be seriously considered in diagnosticating hereditary lues, since there is no good evidence to show that fetal rickets ever occurs and it is agreed that scorbutus is rare before the sixth month of life has been reached.

In passing it is interesting to note that lues of the fetus and newly born child apparently, even in advanced cases, interferes very little, if any at all, with skeletal growth since the fetuses which we have studied were in all respects, as far as careful anthropometry could determine, of normal growth for the age which they had reached.

It is our purpose shortly to publish a series of studies in an attempt to correlate the pathology of the syphilitic bone with the shadow cast by it on exposure to the X-ray.

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* Since this case was under observation the father of the patient has been found to have an aortic aneurysm and is at present under treatment in the out-patient department of The Johns Hopkins Hospital.

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INTERNAL MIGRATION OF THE OVUM

By George W. Corner

(From the Station for Experimental Evolution of the Carnegie Institution, Cold Spring Harbor, Long Island, and the Anatomical Laboratory of The Johns Hopkins University)

In contemplating the passage of fertilized ova from the Graafian follicle to their resting-place in the uterus, observers have often been struck by the fact that an egg discharged from a given ovary may pass through the opposite tube, or when the uterus is bicornate, may actually find lodgement in the opposite cavity. Long tubular bicornate uteri like those of swine offer ready demonstration of migration of the ovum, for it is a very frequent observation indeed (about once in three cases) that one horn of a pregnant uterus contains more embryos, the other less, than the number of corpora lutea in the corresponding ovary. To cite one example of hundreds available, it was found in a certain sow (Fig. 1) that the left ovary contained seven recent corpora lutea, the right ovary two; the left uterine cornu four fetuses and the right five. Three of the embryos in the left uterine cornu had originated, therefore, in the right ovary. Like conditions have long been known in other animals with similar uteri.

Fig. 1.—Ovaries and uterus of pregnant sow in which migration has occurred. Seven corpora lutea in left ovary, two in right ovary; four fetuses in left cornu, five in right cornu. One-fourth natural size.

In the human uterus, with its single cavity, migration of the ovum will not be detected except under peculiar conditions, but numerous clear cases have been described. In many tubal pregnancies the corpus luteum has been found in the ovary opposite to the pregnant tube; for an especially convincing case of this type the reader is referred to one reported by Williams (1917). Migration has also been seen in cases of pregnancy in bicornate uteri, and offers at least a plausible explanation of cases such as those reported by Kelly (1906) and Winckler (1905), in each of which one ovary and the opposite tube were removed, but nevertheless the patients subsequently became pregnant.

The frequency of migration of the ovum in the human subject is not known, but may be crudely estimated by various means. Mayrhofer (1876) considered that migration must occur at least once in 10 ovulations. The present writer (Corner, 1915) found from a study of 128 pregnancies in swine that in this species one or more ova migrate in at least one-third of all cases. An exact statement cannot be made because the data are obscured by non-development of one or more embryos in four-fifths of the litters.

It is obvious, as first pointed out by Kussmaul (1859), that there are two possible pathways of migration. Either the ova pass from the ovary into the abdominal cavity and thence directly into the opposite tube (external migration, Fig. 2,) or they travel by way of the homolateral tube into the uterus and thence to the place of implantation in the contralateral uterine cornu or Fallopian tube (internal migration, Fig. 3). External migration is not possible in those mammals in which the distal portion of the tube forms a closed pouch or ovarian capsule about the ovary, as in the rat (Fig. 4) and guinea-pig; but no such anatomical bar exists in man, rabbit or cat, in which the tubal extremities open freely into the abdominal cavity. In these animals it is conceived that the possibility of external migration is further enhanced by the action of the

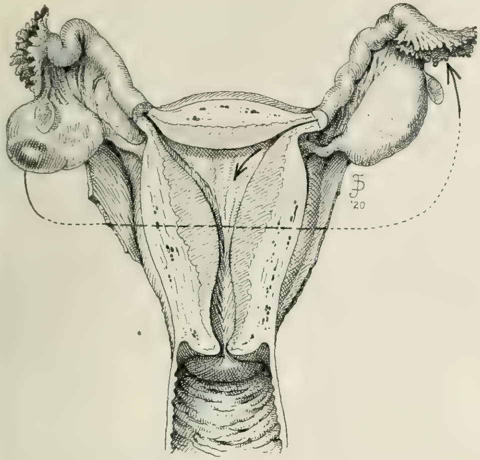

FIG. 2.—Diagram illustrating external migration of the human ovum. Four-fifths natural size.

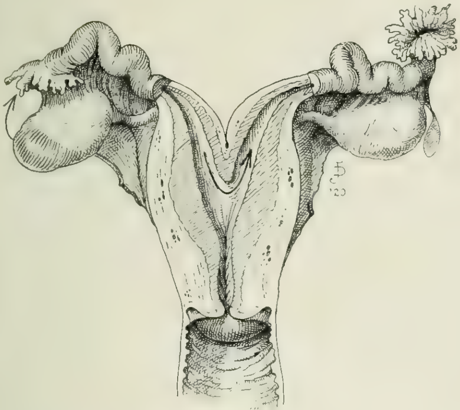

FIG. 3.—Diagram illustrating hypothetical internal migration of the human ovum. Four-fifths natural size.

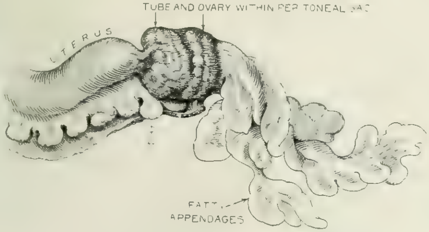

FIG. 4.—Illustrating closed ovarian capsule of Fallopian tube in the rat, which makes external migration of the ovum impossible. × 7.

ciliated lining and possibly by peristaltic movements of the Fallopian tubes, which produce currents in the films of abdominal fluid between the pelvic organs, by which ova may

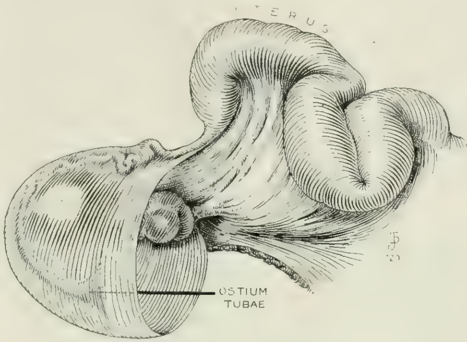

FIG. 5.—Illustrating form of the Fallopian tube of the sow, theoretically permitting migration of the ovum by either route. Two-thirds natural size.

be drawn into the open ostia. Experimental evidence of external migration has been given by Leopold (1880), who excised one tube and the opposite ovary in rabbits, and found the animals still capable of bearing young.

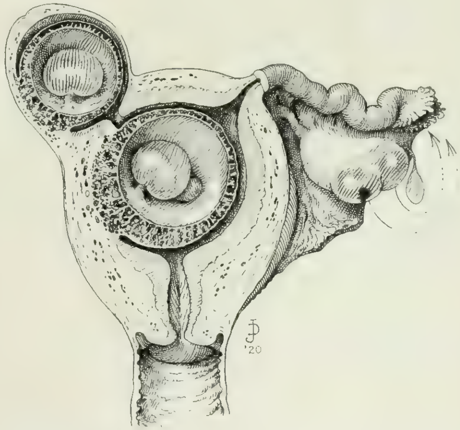

FIG. 6.—Diagram constructed from the verbal description of Andrews' case of supposed internal migration of the human ovum (see text).

With the rarest exceptions, all convincing human cases of migration of the ovum are capable of explanation by the external route. No conclusive evidence, clinical or experimental, has been found to show that internal migration occurs, and thus it has remained merely a hypothetical possibility. Andrews (1912-13), however, has recently reported a case (Fig. 6) which seems to fall into this class, since there was an interstitial pregnancy in the right side which could be excised

without opening the uterine cavity, with a normally implanted twin embryo in the uterine cavity, in a woman whose right tube and ovary were entirely absent.

Undoubtedly, however, much of the supposed evidence for migration from clinical cases has been uncritical or incomplete, so that Burckhard (1904) did not hesitate even to deny the occurrence of migration by either route, except in a few human cases in which there is a previous abnormal arrangement of the pelvic organs. Under this exception he admits some of the cases of tubal pregnancy in the contralateral tube, as described above. In those human and experimental cases in which previous excision of a tube is part of the *mise-en-scène,* he feels that the possibility of subsequently recurring patency of the stump has not been sufficiently considered; and in the study of animals with bicornate uteri and large litters, he believes errors have arisen in consequence of the failure of some of the corpora lutea to develop or to persist, or, on the other hand, because no allowance was made for the possible persistence of corpora lutea from previous ovulations. Although these objections are not all tenable, they must at least be considered in future attempts to demonstrate one or the other kind of migration of the ovum.

With respect to the pig, the present writer has direct information as to the possible causes of error suggested by Burckhard. As before pointed out (Corner 1915), there is no likelihood of confusing corpora lutea of different ovulations, owing to their rapid retrogression; while failure of corpora lutea to develop or to persist during pregnancy has never been observed in a rather large experience. Polyovular follicles undoubtedly occur and may perhaps even attain full development and discharge of their ova, but they are rare, and no experienced student of the ovary could believe them sufficiently numerous to explain away a frequency of migration amounting to one-third or more of all pregnancies. Therefore, as will be confirmed by additional evidence to be given below, in the pregnant sow the number of corpora lutea found in the ovaries probably represents with perfect accuracy the number of eggs which were discharged into the Fallopian tubes at the ovulation which gave rise to the pregnancy.

The Evidence for Internal Migration

In the writer's previous brief discussion of migration of the ovum in swine, to which reference has already been made, it was assumed that the cases were of the external variety, merely because this hypothesis had been proven more likely with regard to the human cases. Küpfer (1920) who has studied seven cases of migration in swine, makes the contrary assumption as to the route. In this guess he is correct, for the statement can now be made that after all, in the pig, external migration has not been demonstrated, but that migration of fertilized ova by the internal route is a frequent phenomenon of physiological significance.

The evidence which not only demonstrated internal migration, but also suggests some of the factors which regulate the process, has been extracted from data gathered (for another purpose) during a detailed examination of the ovaries and uteri of 545 sows. The work was done during a stay at the Station for Experimental Evolution of the Carnegie Institution of Washington, at Cold Spring Harbor, Long Island, and the writer is most grateful to the Director, Dr. C. B. Davenport, for the opportunities afforded. Further thanks are due to Messrs. Joseph Stern and Company, meat packers, of New York City, for material, and to Mr. Clyde E. Keeler for assistance.

TABLE I

Showing Numbers of Corpora Lutea in Ovaries, and of Ova in Fallopian Tubes, in 26 Consecutive Cases of Recent Ovulation in Swine

Serial number	Corpora lutea in left ovary	Corpora lutea in right ovary	Ova in left tube	Ova in right tube	Total corpora lutea	Total ova found	Ova not found
1	3	0	3	0	3	3	..
2	3	5	3	5	8	8	..
3	7	4	7	4	11	11	..
4	3	6	3	5	9	8	1
5	4	5	4	5	9	9	..
6	5	2	5	2	7	7	..
7	5	4	5	3	9	8	1
8	6	3	6	3	9	9	..
9	2	6	2	6	8	8	..
10	4	3	4	3	7	7	..
11	7	5	7	5	12	12	..
12	4	6	4	6	10	10	..
13	5	3	4	2	8	6	2
14	4	2	4	2	6	6	..
15	11	0	11	0	11	11	..
16	6	2	6	2	8	8	..
17	5	3	5	3	8	8	..
18	8	1	7	1	9	8	1
19	5	6	5	6	11	11	..
20	4	3	4	3	7	7	..
21	8	2	7	2	10	9	1
22	4	5	4	5	9	9	..
23	4	4	4	4	8	8	..
24	4	3	4	3	7	7	..
25	5	3	4	3	8	7	1
26	5	3	5	3	8	8	..
Totals	131	89	127	86	220	213	7

Twenty-six consecutive sows were selected, by the appearance of the ovaries, as having ovulated within the past three days; the ova were therefore en route through the Fallopian tubes, and were actually recovered therefrom, by a method previously described (Corner and Amsbaugh, 1917). By using great care in the procedure, and washing out each tube as often as

five times when necessary, it was found possible to collect from a given sow, with almost mathematical certainty, all the ova expected on the basis of the corpus luteum count. In all 26 sows examined, there was a total of 220 corpora lutea, against which 213 ova were regained from the oviducts, or 96 per cent of those expected (Table I).

TABLE II

Showing Distribution of Embryos in Uterus, When Equal Numbers of Ova Were Discharged from Each Ovary

Serial number	Corpora lutea in left ovary	Corpora lutea in right ovary	Embryos in left cornu	Embryos in right cornu	Result
27	4	4	4	4	
28	6	6	6	6	
29	4	4	4	4	
30	3	3	3	3	
31	4	4	4	4	
32	5	5	5	5	
33	5	5	5	5	
34	4	4	4	4	
35	4	4	4	4	No migration.
36	3	3	3	3	
37	3	3	3	3	
38	5	5	5	5	
39	3	3	3	3	
40	4	4	4	4	
41	4	4	4	4	
42	3	3	3	3	
43	3	3	3	3	
44	3	3	4	2	
45	3	3	4	2	Migration of one ovum in each case.
46	4	4	5	3	
47	7	7	8	6	

The reader will have noticed that not only practically all the discharged ova are readily discoverable, but also that the eggs in a single tube are always accounted for by the discharged follicles (corpora lutea) in the corresponding ovary. In these 26 consecutive cases, then, there was no case of external migration.

Next a similar table was made by counting the corpora lutea and the embryos of nearly 500 pregnant sows which were passing through the same abattoir. In these animals, in spite of the fact already mentioned, that a large early embryonic mortality obscures and lowers the apparent proportion of migration, still (as was found some years ago) about one-third of the sows show migration of one or more ova. If distributed, for the sake of comparison with the first series, into groups of 26, no such group contains less than five cases in which migration occurs.

These two facts, that migration in general is very common, but external migration rare or non-existent, together prove the occurrence of internal migration of the fertilized ovum of the sow.

Determining Factors and Probable Utility of Internal Migration of the Ovum

We may now venture the hypothesis that the phenomenon of internal migration has a practical utility in the pig; it is important that the individual embryos of the large litters shall each find a fair share of space in the uterine cavity. As

TABLE III

Showing Distribution of Embryos in Uterus, When the Total Number of Ova Discharged was Uneven, One Ovary Exceeding the Other by One Ovum

Serial number	Corpora lutea in left ovary	Corpora lutea in right ovary	Embryos in left cornu	Embryos in right cornu	Result
48	5	4	5	4	
49	4	3	4	3	
50	3	4	3	4	
51	4	3	4	3	
52	4	5	4	5	
53	5	6	5	6	
54	5	4	5	4	No migration.
55	4	5	4	5	
56	3	4	3	4	
57	4	3	4	3	
58	3	4	3	4	
59	4	3	4	3	
60	3	2	3	2	
61	3	4	3	4	
62	6	5	5	6	
63	5	6	6	5	
64	4	5	5	4	
65	3	4	4	3	
66	3	2	2	3	Migration of one ovum.
67	3	4	4	3	
68	5	4	4	5	
69	3	4	4	3	
70	3	4	4	3	
71	2	3	4	1	Migration beyond balance.

shown by the examples in Table I, the right and left ovaries often discharge very unequal numbers of ova, which might well lead, were it not for migration, to the overcrowding of embryos in one cornu.

This suggestion was tested by analysis of the records of the pregnant uteri, from the whole series only those cases, 113 in

number, being chosen, in which all the corpora were represented by normally implanted embryos, thus avoiding the obscuring effect of early embryonic degeneration. If migration is by rule and not by chance, then in these cases we should find: (1) that when the ovaries discharge equal numbers of ova, the number of embryos in each chamber remains equal; (2) however unequal the output of the two ovaries, the number of embryos in the uterine cornua should tend to approach equality. It will also be clear that cases which deviate from these expectations should be commonly those in which the litters are small, and further that they will be damaging to the hypothesis in proportion to the number of ova which in any one case migrate in the adverse direction.

I. In 21 cases the ovaries discharged equal numbers of eggs. By hypothesis there should have been no migration. Result: no migration in 17 cases, but in four cases there was migration of one ovum in each (Table II).

II. In 24 cases the total number of eggs discharged was uneven, one ovary exceeding the other by one egg. In this circumstance, by our hypothesis it is a toss-up whether or not one ovum shall migrate, as the balance cannot be perfectly restored. Result: no migration in 14 cases; migration of one ovum in 9 cases; migration beyond balance in one case; migration toward excess side, no case (Table III).

III. In 68 cases, the output of one ovary exceeded that of the other by two or more eggs. By hypothesis there should have been migration toward the lesser side in all cases. Result: in 21 cases perfect balance was obtained by migration; in 20 more, the total number of eggs being uneven, balance was restored to $\pm\frac{1}{2}$. In 3 cases, in all of which the discrepancy between the ovaries was great, perfect balance was not quite attained in the uterus (Cases 113-115). In 9 cases migration did not stop at an even balance; in 7 of these one ovum too many went over, in 2 cases two ova migrated beyond the balance, in no case, however, forcing more than seven embryos into the uterine chamber. In 14 cases there was no migration at all, but in all these the original imbalance was small in degree, so that the need of migration was slight. In one case there was migration of one ovum toward the excess side (Case 139, Table IV).

To sum up, the hypothetical expectation was fulfilled in 84 of 113 cases, with 29 deviations, all of which were trivial in degree; hence the supposition that the process of internal migration is under physiological regulation for useful ends seems to be justified.

The anatomical mechanism of internal migration is now open to conjecture and experiment, but there can be but little doubt that the embryos are shifted by the peristaltic action of the uterine musculature. Some such action must be postulated in any case to account for the regular spacing of the implanted embryos within the uterus; and the only novelty in the present contribution is the notion that both cornua act as one continuous tube, which must be readily capable of peristalsis in either direction. It may well be also that the peculiar form of the ungulate blastodermic vesicle, which reaches the length of 30 or more centimeters before implantation, may render it espe-

TABLE IV

SHOWING DISTRIBUTION OF EMBRYOS IN UTERUS, WHEN THE OUTPUT OF THE OVARIES WAS UNEQUAL, ONE OF THE OVARIES EXCEEDING THE OTHER BY TWO OR MORE OVA

Serial number	Corpora lutea in left ovary	Corpora lutea in right ovary	Embryos in left cornu	Embryos in right cornu	Result
72	4	6	5	5	Migration to exact balance.
73	5	7	6	6	
74	6	4	5	5	
75	4	6	5	5	
76	8	0	4	4	
77	5	3	4	4	
78	6	4	5	5	
79	2	4	3	3	
80	6	4	5	5	
81	5	1	3	3	
82	8	2	5	5	
83	2	6	4	4	
84	1	3	2	2	
85	4	2	3	3	
86	1	5	3	3	
87	8	0	4	4	
88	5	1	3	3	
89	4	2	3	3	
90	6	2	4	4	
91	3	1	2	2	
92	5	1	3	3	
93	5	2	4	3	Total number of ova uneven; balance restored to $\pm\frac{1}{2}$.
94	4	1	2	3	
95	3	6	5	4	
96	7	2	4	5	
97	6	3	4	5	
98	4	7	5	6	
99	6	1	4	3	
100	5	0	3	2	
101	2	5	3	4	
102	1	8	5	4	
103	3	6	4	5	
104	2	7	5	4	
105	1	4	3	2	
106	4	1	3	2	
107	4	1	3	2	
108	4	1	2	3	
109	2	5	3	4	
110	8	5	7	6	
111	8	1	4	5	
112	5	2	3	4	
113	11	0	8	3	Balance not fully restored.
114	7	0	5	2	
115	1	9	4	6	
116	2	8	6	4	Migration beyond balance.
117	6	0	2	4	
118	4	7	7	4	
119	9	3	5	7	
120	6	2	3	5	
121	7	4	4	7	
122	2	6	5	3	
123	4	0	1	3	
124	5	3	3	5	
125	5	3	5	3	No migration.
126	6	4	6	4	
127	5	3	5	3	
128	5	3	5	3	
129	2	4	2	4	
130	6	4	6	4	
131	4	6	4	6	
132	4	6	4	6	
133	2	5	2	5	
134	4	2	4	2	
135	3	1	3	1	
136	3	5	3	5	
137	1	4	1	4	
138	5	3	5	3	
139	6	7	5	8	Migration toward excess side.

cially suitable for transportation by the uterus. It will be of much interest to learn whether migration of the pig's ovum can be demonstrated before the tenth day, while it is still minute and spherical.

If the forgoing discussion has any bearing upon the clinical side of the question, it is that internal migration of the human ovum is still to be regarded as occasionally possible, more especially perhaps in cases of multiple gestation. Twin embryos were present in the case of Andrews, previously mentioned, which is up to the present the most satisfactory case among those interpreted as examples of this form of migration in the human.

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SERIOUS REACTIONS TO REPEATED TRANSFUSIONS IN PERNICIOUS ANEMIA

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Since the introduction of blood transfusion as a therapeutic procedure in the treatment of pernicious anemia, numerous reports have appeared upon the various phases of reactions after transfusion. Three cases are here reported, in which after a large number of transfusions, there occurred extremely severe reactions following the transfusion of apparently compatible blood. Cases I and II of this series have been briefly referred to by Sydenstricker, Mason and Rivers,[1] who concluded that transfusion is a self-limited process in pernicious anemia. In two of the cases the severity of the reaction seems to have been largely responsible for the death of the patient. It was considered inadvisable and dangerous to continue this form of therapy in the other patient, who died a short time after discharge from the hospital.

Blood matching of recipient *vs.* donor was carried out in every instance, and the donor chosen only in complete absence of microscopic agglutination or hemolysis of the donor's cells by the recipient's serum, or the recipient's cells by the donor's serum. The matching was carried out according to the method described by Sydenstricker and others.[1] A small amount of blood is drawn into a Wright pipette or test-tube and centrifuged to obtain the serum. A cell suspension is made by mixing two drops of blood in 5 c. c. of normal salt solution, which may contain 0.125 per cent sodium citrate. Coverslips are passed through 95 per cent alcohol and polished. One drop of serum and one drop of cell suspension are placed on the coverslip by means of capillary tubes, and mixed with a glass rod. The preparation is then inverted on a hollow ground slide, sealed with oil, and examined. In several instances early in the series of transfusions the incubation period was only 30 minutes. It was noted in Transfusion V of Case I that agglutination did not occur at the end of 30 minutes, but did occur at the end of 45 minutes. Consequently, later matchings were incubated for one hour or longer. In every instance in which the donor had given blood for an earlier transfusion he was rematched against the blood of the recipient before being used again, for it has been shown by McClure and Dunn[2] that a donor may be found compatible for one transfusion, but may be incompatible for a subsequent transfusion and this can be demonstrated by blood matching.

The early transfusions of the series were carried out by the Lindemann syringe-cannula method.[3] In the later transfusions the citrate method described by Lewisohn[4] was used.

This series of cases is reported chiefly to show the inadequacy of the present method of determining the compatibility of blood for transfusion, particularly in those cases in which the recipient has been the subject of a large number of earlier transfusions.

CASE I

FIRST ADMISSION

J. B. Female, *aet.* 43, white, married, occupation housewife. Medical No. 34717. *Diagnosis:* Pernicious anemia. Transfusion of blood. Reaction following blood transfusion. Admitted: September 10, 1915.

The *complaint* was weakness, shortness of breath and increasing pallor.

The family history was negative.

Past History.—The patient had scarlet fever at 6. She had had two miscarriages. During the past two years her periods had been more profuse than normal. There had been diarrhea at times.

Present Illness.—For over a year the patient had been troubled with burning on urination, a scalded and burning sensation in her mouth, and a chronic cough. For the past ten months she had suffered from lightheadedness, and for the past two months roaring in her ears. She had noticed a yellow coloration of the skin, numbness and tingling in her fingers and toes, palpitation and dyspnœa on exertion, œdema of her feet recently, and dimness of vision at times.

The physical examination showed a general pallor with sallowness of the complexion and an icteric tint to the sclerotics and skin, striking pallor of the mucous membranes, slight diffuse enlargement of the thyroid, bleeding from the gums, and retinal hemorrhages. The relative cardiac dulness measured 14 cm. by 3.5 cm., and there was a blowing systolic murmur at the apex. The liver extended one finger's breadth below the costal margin on inspiration, and the spleen was palpable at the costal margin. There was slight pitting œdema of the ankles. The blood pressure was 140/95. The patient showed an almost constant elevation of temperature from 100° to 102° F.

Laboratory.—Hb. (Sahli) = 27%. R. B. C. = 1,136,000. W. B. C. = 3,560. The stained smear showed a typical picture as demonstrated in the charts. Gastric analysis showed a hydrochloric acid deficit of 8%, and a combined acid of 2%. The Wa. R. was negative. The urine showed a trace of albumin and a few W. B. C.

TRANSFUSIONS

I. Eight hundred cubic centimeters of matched whole blood given during 30 minutes by the Lindemann method. Upon returning to the ward the patient experienced a generalized itching sensation over her body; the skin was somewhat flushed and an urticarial eruption developed. She vomited once. There was slight temperature elevation. Donor, H. E. B., husband.

II. Nine hundred cubic centimeters of matched whole blood given during 30 minutes. Lindemann method. No reaction. Donor, J. A. D.

III. Nine hundred cubic centimeters of matched whole blood. Lindemann method. Upon returning to the ward the patient complained of itching all over and vomited once. No other reaction. Donor, H. W. D.

IV. Nine hundred cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, M. E. B.

V. The donor's and recipient's blood had been incubated and read at the end of 30 minutes for matching; there was no agglutination or hemolysis. Twenty cubic centimeters of blood were withdrawn from the donor and injected into the vein of the recipient; the latter had been feeling perfectly well. Almost instantly the patient's face became flushed and she complained of feeling badly and aching all over, and especially of pain in her arms and back. Her respirations became more rapid and there was slight cyanosis; she complained of a choking sensation and coughed several times. No more blood was injected. The symptoms became slowly more marked and the patient attempted to vomit several times. Transfusion was abandoned. Upon returning to the ward the patient had a severe chill lasting 40 minutes and a transient temperature elevation to 102° F. The pulse was weak and variable. Urine voided five hours later gave a positive test for Hb. The blood of the recipient and of the donor were rematched; at the end of 30 minutes the preparations were negative, but at the end of 45 minutes, in thick preparations, there was marked agglutination of the recipient's red blood cells by the serum of the donor. Thin preparations were negative at the end of one hour. Four hours following this reaction the patient felt perfectly well. Donor C. W. D.

VI. Nine hundred cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, husband.

X-30-15. The patient was discharged. Hb = 62%. R. B. C. = 4,000,000.

SECOND ADMISSION

Medical No. 35134. *Diagnosis:* Pernicious anemia. Transfusion of blood. Admitted: December 6, 1915. The *complaint* was weakness.

During the previous three weeks the patient had been troubled with palpitation and pounding in her ears, dyspnœa on exertion and edema of the ankles at night. She entered the hospital for an examination of her blood.

The *physical examination* was as upon the previous admission. In addition, there were fresh retinal hemorrhages. Her temperature ranged from 99.8° to 100.6° F. up to the time of the first transfusion, after which it remained normal. Blood: Hb. (Sahli) = 29%. R. B. C. = 1,148,000. W. B. C. = 4,000. Resistance of R. B. C. to hypotonic salt solution (fragility test): Hemolysis began at 0.40% and was complete at 0.25%. The urine showed a very faint trace of albumin, and a positive test for urobilin.

TRANSFUSIONS

VII. Seven hundred and eighty-eight cubic centimeters of matched whole blood. Lindemann method. The patient became restless, but suffered no reaction. Donor, J. A. D., brother.

VIII. Nine hundred and two cubic centimeters of matched whole blood. Lindemann method. The patient complained that her eyelids felt swollen, and of slight itching in the palms of her hands. No other reaction. Donor, C. W. D.

IX. Eight hundred cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, husband.

XII-21-15. The patient was transferred to the Surgical Department for splenectomy. Hb. = 60%. R. B. C. = 3,432,000.

SURGICAL ADMISSION

Surgical No. 38909. *Diagnosis:* Pernicious anemia. Admitted December 21, 1915.

XII-21-15. Splenectomy was performed. There was a slight oozing of blood from the operative wound which ceased with a transfusion of 286 cubic centimeters of blood.

TRANSFUSIONS

X. Two hundred and eighty-six cubic centimeters of matched whole blood. Lindemann method. No reaction. Donors, A. D., brother, and B., husband.

XI. Five hundred and twenty cubic centimeters of matched whole blood. Lindemann method. No immediate reaction. Upon returning to the ward the patient complained of itching of her back which showed an urticarial eruption. Her pulse went to 140 per minute and her respirations to 40. There was a chill, lasting about 30 minutes, and the patient became quite cyanotic. There was a transient elevation of temperature to 101.5°. The reaction lasted one hour. Donor, S. I. L.

XII. Five hundred and fifty cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, not stated.

XIII. Six hundred and sixty cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, husband.

XIV. Two hundred and seventy cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, W.

II-15-16. There was an uneventful recovery from splenectomy. The patient was discharged. Hb. = 82%.

THIRD ADMISSION

Medical No. 36344. *Diagnosis:* Pernicious anemia. Transfusion of blood. Admitted: August 2, 1916.

The patient had been in fair condition while at home; she had rested a great deal and spent half of each day in bed. She had recently been growing gradually weaker, and there had been edema of the ankles when she was up out of bed.

The *physical examination* was the same as upon previous admissions. In addition, there was slight edema of the ankles, and a splenectomy scar in the left abdomen. There was a constant elevation of temperature ranging from 100° to 102°. Hb. = 24%. R. B. C. = 1,238,000. W. B. C. = 9,250. The urine showed a faint trace of albumin and a positive guaiac test for hemoglobin.

TRANSFUSIONS

XV. Three hundred and fifty cubic centimeters of matched whole blood. Citrate method. The blood was given slowly during one hour. Twenty minutes after the procedure, the patient had a severe shaking chill, the temperature rising rapidly to 106° F., and falling gradually. There was no hemoglobinuria on repeated examinations. This transfusion was held very badly and the hemoglobin and red blood cell count continued to drop. Donor, B., husband.

XVI. Five hundred and fifty cubic centimeters of matched whole blood. Citrate method. During this operation a great deal of trouble was experienced from the blood clotting in the needle; the clotting seemed to take place immediately upon contact between the donor's and the recipient's blood. This difficulty was overcome by adding twenty-five cubic centimeters of sodium citrate. There was an immediate severe reaction with a shaking chill lasting about twenty minutes, an elevation of temperature to 106.6° F. and nausea. The temperature fell gradually to its former level. There was a marked hemoglobinuria which persisted until the time of death. There had been no untoward symptoms after the injection of the first twenty cubic centimeters of blood. Donor, D., brother.

VIII-14-16. The patient had grown progressively weaker, and showed no improvement in the blood picture. Inasmuch as better results had been obtained on previous admissions with the Lindemann method of transfusion, it was decided to make use of this procedure.

XVII. Seven hundred cubic centimeters of matched whole blood. Lindemann method. Twenty cubic centimeters of blood were given and after a wait of ten minutes there had been no reaction. The remainder of the blood was given in about forty minutes. The patient had no reaction while on the table, but on the way to the ward she developed a severe shaking chill, lasting about thirty minutes, with an elevation of temperature to 104° F. This was accompanied by marked cyanosis, without any demonstrable signs of cardiac dilatation, dyspnœa, pain in the back, and marked general discomfort. Subjectively, this was the severest reaction which the patient had had. There was marked hemoglobinuria; the urine was almost black. Seven hours later the patient was a little more com fortable but still in a critical condition with a temperature of 103.6° F. and a pulse of 160, weak in quality. Donor, T. A. L.

VIII-15-16. The patient had been irrational all day with a temperature of 102.4° F. and a pulse of 130, of poor quality. Strophanthin, Murphy drip per rectum, and subcutaneous infusion of normal salt solution produced no improvement; she had to be catheterized, and 300 cubic centimeters of black urine were obtained, showing a strongly positive guaiac reaction, but no casts or red blood cells.

VIII-17-16. There was still a marked hemoglobinuria in catheterized specimens of urine. The patient died at 6.45 a. m.

MACROSCOPIC MATCHINGS WITH VARIOUS DILUTIONS OF DONOR'S AND RECIPIENT'S R. B. C. AND SERA

	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	c. c.	
Recipient's serum	1/4	1/4	1/4	1/4	1/4	1/4	1/4	No agglutination
Donor B., R. B. C.—50%	1/4	1/8	1/16	1/32	1/64	1/128	1/256	or hemolysis.
Recipient's serum	1/4	1/4	1/4	1/4	1/4	1/4	1/4	No agglutination
Donor D., R. B. C.—50%	1/4	1/8	1/16	1/32	1/64	1/128	1/256	or hemolysis.
Recipient's serum	1/4	1/8	1/16	1/32	1/64	1/128		No agglutination
Donor B., R. B. C.—10%	1/4	1/4	1/4	1/4	1/4	1/4		or hemolysis.
Recipient's serum	1/4	1/8	1/16	1/32	1/64	1/128		No agglutination
Donor D., R. B. C.—10%	1/4	1/4	1/4	1/4	1/4	1/4		or hemolysis.

CASE I

Date	Hb.	R. B. C.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. M.	L. M.	Tr.	Myelocytes	Anisocytosis	Poikilocytosis	Punctate basophilia	Diffuse basophilia	Nucleated R. B. C.	Macrocytes	Microcytes	Platelets	Vital staining R. B. C.	Transfusions
IX-10-15	27%	1,136,000	3,560	66.0	.5	.5	27.5	3.0	1.0	1.5	+	+	—	—	0	+	+	..	..	....
IX-24-15	20	680,000	..	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	9-25-15 800 c. c.
IX-29-15	35	1,664,000	2,160	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	9-30-15 900 c. c.
X- 9-15	55	2,240,000	3,280	52.5	.5	0	42.0	3.5	0	1.5	..	..	..	..	1.0	..	..	..	..	10- 9-.5 900 c. c.
X-15-15	50	3,360,000	....	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	10-16-15 900 c. c.
X-18-15	55	4,170,000	....	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	10-23-15 20 c. c.
X-24-15	52	2,672,000	....	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	10-30-15 900 c. c.
X-31-15	62	4,000,000	....	70.0	1.6	.4	20.0	3.6	1.6	2.8	0	..	..	..	..	..	..	..	..	....
XII- 6-15	29	1,148,000	4,000	56.5	.5	0	41.0	1.5	.5	0	+	+	+	+	0	+	-	..	..	Second admission. 12-10-15 788 c. c.
XII-14-15	42	2,450,000	3,100	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	12-14-15 902 c. c.
XII-17-15	52	2,968,000	3,280	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	12-18-15 800 c. c.
XII-19-15	60	3,432,000	....	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	12-21-15 286 c. c. Splenectomy.
XII-22-15	48	3,000,000	7,000	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	12-30-15 520 c. c.
I- 7-16	60	3,024,000	4,760	33.0	0	0	54.5	7.0	3.0	2.5	..	..	..	-	7	..	.	..	..	1-22-16 550 c. c.
II-11-16	74	3,208,000	6,080	53.5	2	.5	23.5	13.5	4.0	0	..	..	..	..	1.0	..	..	..	..	2-12-16 660 c. c. 2-13-16 270 c. c.
II-15-16	82	......	....	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	....
VIII -2-16	24	1,238,000	9,250	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	Third admission. 8- 3-16 350 c. c.
VIII- 4-16	30	1,560,000	....	43.5	1.0	.5	52.5	.5	.5	1.5	+++	+++	+	+	57	..	..	..	..	....
VIII- 7-16	28	1,112 000	....	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	8- 9-16 550 c. c
VIII-12-16	26	1,544,000	....	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	....
VIII-15-16	25	1,048,000	10,400	....	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	8 15 16 700 c. c.
VIII-16-16	24	......	19,400	60.0	..	..	....	....	..	..	..	..	..	..	..	..	..	..	..	....

Analysis.—This patient (Case I) had three admissions to the hospital during a period of 11 months, and received a little more than 10¾ liters of blood in 17 transfusions. Eight times during this series she experienced reactions of varying degrees of severity, showing a marked hemoglobinuria on three occasions. Transfusion V gave one of the most severe reactions accompanied by hemoglobinuria, although only 20 c. c. of blood were injected. The same donor seems to have been used for the eighth transfusion, which was followed by no reaction except pruritus and discomfort about the eyelids. The patient's husband acted as donor six times, giving a total of 3,620 c. c. of blood, with one mild reaction, after Transfusion I, shown by urticaria and pruritus, and with one severe reaction, after Transfusion XV, shown by a severe shaking chill and an elevation of temperature to 106° F., but no hemoglobinuria; on four occasions his blood produced no reaction of any sort. Splenectomy gave no permanent benefit. After the reactions to Transfusions XV and XVI macroscopic matchings of the recipient's serum against the donor's cells were carried out with varying dilutions of serum and cell suspension; they were all negative for agglutination or hemolysis.

CASE II

First Admission

H. A. P. Male. *Aet.* 62. White, widowed; occupation—oiler of machinery. Medical No. 35335. *Diagnosis:* Pernicious anemia. Admitted: January 3, 1916.

The *complaint* was weakness and lack of blood.

Family History.—One brother had died of cancer of the rectum; one sister had died of tuberculosis. Otherwise, negative.

Past History.—The patient's general health had been good. He had had recurrent attacks of malaria from 10 to 50 years of age; had always worked hard, 11 to 13 hours daily, exposed to high temperatures. Otherwise negative.

Present Illness.—The symptoms were of one year's duration. He had first noticed frequency of urination; later, weakness in his knees, and then the entire body, which had become progressively worse. The weakness was accompanied by shortness of breath. During the three weeks before admission there had been precordial pain on exertion, and he had become too weak to work. He had suffered from anorexia and constipation and had vomited on one occasion. There was early loss of sexual power. He had lost 37 pounds in weight since the onset of his illness.

The *physical examination* revealed dyspnœa on slight exertion, a lemon tint of the skin, a flabby musculature, and evidence of loss of weight, but with a still thick layer of subcutaneous fat. The right pupil was larger than the left. There was marked oral sepsis, and the tongue showed papillary atrophy. A faint systolic murmur was heard at the apex of the heart. The blood pressure was 118/60. There was slight edema of the ankles. The fundi showed numerous retinal hemorrhages. There was a constant elevation of temperature ranging from 99° to 101° F.

Laboratory.—Blood: Hemoglobin (Sahli) = 26%. R. B. C. = 936,000. W. B. C. = 5,440. Gastric analysis showed no free acid. The urine showed albumin and granular casts. The Wa. R. was negative.

TRANSFUSIONS

I. Two hundred cubic centimeters of whole blood. Lindemann method. The patient had a severe chill with signs of collapse and an elevation of temperature to 102.8° F. There was no hemoglobinuria. Donor, E. M. P.

II. Three hundred cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, A. P.

III. Six hundred and eighty-two cubic centimeters of matched whole blood. Lindemann method. No reaction except an elevation of temperature to 101° F. Donor, L. P.

IV. Six hundred and forty cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, C. N. P.

V. Six hundred and thirty-eight cubic centimeters of matched whole blood. Lindemann method. The patient felt chilly after the transfusion, but there was no rise in temperature. Two urticarial wheals developed, and the patient vomited once. No other reaction. Donor, H.

VI. Six hundred and sixty cubic centimeters of matched whole blood. Lindemann method. The patient felt chilly and his temperature rose to 101.6° F. No other reaction. Donor, W. B. P.

II-8-16. Hb. = 65%. R. B. C. = 4,680,000. W. B. C. = 1,400.

II-12-16. Transferred to the Surgical Clinic for splenectomy.

Surgical No. 39006. II-12-16. Splenectomy was performed.

VII. Four hundred and forty cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, Y.

VIII. Three hundred and fifty cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, E. P.

II-28-16. Hb. = 80%. R. B. C. = 3,170,000. W. B. C. = 7,200.

II-29-16. The patient was transferred to the Medical Service. There was an uneventful recovery from the operation.

Medical No. 35677. IX. Five hundred cubic centimeters of matched whole blood. Lindemann method. Several hours later the patient complained of a slight chill and his temperature rose to 102° F. Many large urticarial wheals appeared over the entire body, but there were no other symptoms and no hemoglobinuria. Donor, W. E. K.

X. Four hundred and fifty cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, P.

XI. Four hundred and fifty cubic centimeters of matched whole blood. Lindemann method. No reaction. Donor, brother, who had given blood once previously.

IV-19-16. The patient was discharged. Hb. = 81%. R. B. C. = 3,170,000. W. B. C. = 2,800.

Second Admission

Medical No. 36290. *Diagnosis:* Pernicious anemia; combined sclerosis. Admitted, 6-22-1916.

After leaving the hospital the patient gained 27 pounds in weight. There had been shortness of breath on exertion, palpitation, and roaring in the ears, difficulty in holding his urine if the bladder was full, frequency, and nycturia, 3 to 4 times a night. He had become very ataxic and had to use a cane in walking, and there was marked parasthesia of the feet and legs, extending as high as the umbilicus at times, and slight parasthesia of the hands and forearms.

The *physical examination* was the same as upon previous admission. In addition, there was weakness of the leg muscles, marked ataxia, a positive Romberg, loss of muscle sense in the toes, and the knee-kicks and ankle-jerks could not be obtained.

Hb. = 26%. R. B. C. = 1,136,000. W. B. C. = 7,240.

TRANSFUSIONS

XII. Five hundred cubic centimeters of matched whole blood. Citrate method. No reaction. Donor, L. C. P.

XIII. Five hundred cubic centimeters of matched whole blood. Citrate method. No reaction. Donor, C. P.

XIV. Three hundred and fifty cubic centimeters of matched whole blood. Citrate method. There was an immediate reaction with a severe shaking chill lasting 15 minutes, and a transient rise of temperature to 104° F. Nausea and vomiting occurred, and a wheezing respiration. There was no hemoglobinuria. Donor, J. P. Five hours later the patient felt fine.

XV. Four hundred cubic centimeters of matched whole blood. Citrate method. No reaction. Donor, P. This donor had been used twice previously.

XVI. Five hundred and fifty cubic centimeters of matched whole blood. Citrate method. There was a slight chill and an elevation of temperature to 101° F., but no other symptoms. Donor, H.

XVII. Five hundred and fifty cubic centimeters of matched whole blood. Citrate method. No reaction. Donor, P.

VIII-9-16. The patient was discharged.

Hb. = 60%. R. B. C. = 3,500,000. W. B. C. = 3,460.

THIRD ADMISSION

Medical No. 36839. *Diagnosis:* Pernicious anemia; combined sclerosis. Admitted: November 11, 1916.

Complaint the same as upon previous admission. There were no new complaints, but the patient felt somewhat weaker.

The *physical examination* was the same as upon the previous admission and in addition there was slight ataxia of the hands.

Hb. = 24%. R. B. C. = 1,296,000. W. B. C. = 9,600.

TRANSFUSIONS

XVIII. One hundred and fifty cubic centimeters of matched whole blood. Citrate method. There was no reaction except a slight rise in temperature. Donor, son.

XIX. One hundred and twenty cubic centimeters of matched whole blood. Citrate method. There was a very severe reaction with profuse sweating, nausea, and vomiting, and an elevation of temperature to 101.8° F. The patient became very dull and irritable, and his pulse became rapid and of poor quality. His condition was critical for several hours. There was no hemoglobinuria. Donor, son.

XX. One hundred and fifty cubic centimeters of matched whole blood. Citrate method. There was a sharp reaction 45 minutes later with a chill, nausea, and vomiting, and an elevation of temperature to 101.8° F. There was no hemoglobinuria. Donor, brother.

XII-6-16. Further transfusion was abandoned as being too dangerous. The patient was discharged.

Hb. = 18%. R. B. C. = 1,141,000.

XII-18-16. The patient died at home.

Analysis.—This patient (Case II) had three admissions to the hospital in a period of two years, and received a total of a little more than eight and one-half liters of blood in 20 transfusions. He suffered nine reactions of varying degrees of severity. On no occasion was hemoglobinuria demonstrable. During the third admission the patient's son acted as the donor for small transfusions on two occasions; from the first transfusion there was practically no reaction, while after the second reaction the recipient's condition was critical. Donor, W. B. P., was used three times; the use of his blood was followed once by chilly sensations and a slight elevation of temperature, and twice it produced absolutely no reaction. Splenectomy produced no permanent improvement in the patient's condition.

CASE III

FIRST ADMISSION

D. W. Female, *aet.* 45, white, single, occupation—none. Medical No. 38864. Admitted: July 21, 1917. *Diagnosis:* Pernicious anemia, oral sepsis, rectal polyp.

The *complaint* was anemia.

The *family history* was negative.

CASE II

Date	Hb.	R. B. C.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. M.	L. M.	Tr.	Myelocytes	Anisocytosis	Poikilocytosis	Punctate basophilia	Diffuse basophilia	Nucleated R. B. C.	Macrocytes	Microcytes	Platelets	Vital staining R. B. C.	Transfusions
I- 3-16	26%	936,000	5,440	50.	.4	.4	28.8	2.8	.8	13.6?	++++	++	0	+++	+	—		—	—	1-11-16 200 c. c.
I-20-16	—	—	3,860	36.	.4	1.6	38.8	19.6	2.0	.0	++	++	—	++	10	....	....	......	..	1-21-16 300 c. c.
I-22-16	26	1,448,000	....	..	..	..	..	..	..	..	....	....	..	..	..	....	....	......	..	1-24-16 682 c. c.
I-25-16	36	2,352,000	....	..	..	..	..	..	..	..	....	....	..	..	..	....	....	......	..	1-26-16 640 c. c.
I-30-16	50	2,416,000	1,440	54.8	1.2	.4	26.8	14.0	.4	.4	++++	++++	..	+	4	...	....	......	..	2- 2-16 638 c. c.
II- 7-16	54	3,040,000	—	48.0	.8	.0	34.4	14.4	1.6	.8	++	+	—	—	6	....	....	......	..	2- 7-16 660 c. c.
II- 8 16	65	4,680,000	1,400	..	..	..	..	..	..	..	....	....	..	..	..	....	....	.....	..	2-12-16 Splenectomy
II-14-16	47	2,376,000	4,800	72.8	2.4	.4	18.0	2.8	2.4	..	+++	+++	..	..	0	....	....	......	..	2-19-16 440 c. c.
II-21-16	63	2,726,000	1,300	54.4	1.3	2.0	26.6	4.6	4.6	.0	+−	−	..	+	7	....	....	......	..	2-26-16 350 c. c.
II-28-16	80	3,170,000	7,200	..	..	..	..	..	..	..	....	....	..	..	..	....	....	......	..	3- 6-16 500 c. c.
III- 7-16	76	3,824,000	6,200	80.2	1.2	1.2	12.4	2.8	1.2	.0	++	+	0	0	2	....	....	...	..	3-13-16 450 c. c.
IV- 2-16	79	3,000,000	3,160	41.6	1.2	.4	48.8	9.2	1.2	.4	+++	−+	0	0	4	....	....	Very few.	..	4- 5-16 450 c. c.
IV-19-16	81	3,128,000	2,840	32.0	2.0	.4	60.0	3.6	.4	..	++++	−	0	0	4	....	....	Very few.	..	Second admission.
VI-22-16	26	1,136,000	7,240	21.0	2.0	1.0	57.0	8.0	4.0	4.0	++++	−	+	−	216	++++	−−−+	Decr'd.	..	6-28-16 500 c. c.
VI-29-16	30	1,450,000	1,760	30.0	1.0	1.0	56.0	2.0	1.0	.0	++−	+−+	+++	++−	76	....	...	......	..	7- 4-16 500 c. c.
VII- 5-16	38	2,152,000	3,520	..	..	..	..	..	..	..	....	....	..	..	..	....	....	......	..	7- 6-16 350 c. c.
VII- 7-16	45	2,480,000	—	40.0	.0	.0	34.0	3.0	1.0	5.0	....	....	+	+	16	+	....	Decr'd.	..	7-15-16 400 c. c.
VII-16-16	52	3,240,000	—	..	..	..	..		..	..	....	....	..	..	.	....	....	.	..	7-22-16 550 c. c.
VIII- 3-16	50	2,606,000	....	..	..	..	..	..	..	..	....	....	..	..	..	....	....	.....	..	8- 5-16 550 c. c.
VIII- 9-16	60	3,500,000	....	..	..	..	..	..	..	..	....	....	..	..	..	....	....	......	..	Third admission.
XI-12-16	24	1,296,000	4,120	44.0	2.0	1.0	4.0	38.0	2.0	9.0	++	++	++	++	133	.	....	Many.	..	11-14-16 150 c. c.
XI-24-16	20	1,420,000	3,444	20.0	6.0	1.0	7.0	63.0	1.0	2.0	+	+	+	+	..	....	....	.....	..	11-15-16 120 c. c.
XII- 4-16	18	1,040,000	5,000	53.0	3.0	1.0	8.0	29.0	4.0	1.0	+	+	+	+	..	....	....	......	..	12- 3-16 150 c. c.

Past History.—Her general health had been good. There had been intermittent attacks of malaria for several years which finally cleared up with treatment. There had been slight bleeding from hemorrhoids, and she had had nycturia, sometimes once a night. She had had an acute attack of cystitis several years previously, which had cleared up with treatment. Otherwise negative.

Present Illness.—In February, 1917, the patient noticed gradually increasing weakness, and later suffered with pounding of her heart accompanied by asthenia. She had had occasional attacks of vomiting, productive of a large quantity of green fluid; later these vomiting attacks had become very frequent. In April the patient was told that she had anemia and that her hemoglobin was 40%. There had been no diarrhea, but the stools had been more fluid than normal. The patient noticed that her face was pale and that she had a lemon yellow tint to the skin of her body.

Physical Examination.—The general appearance was typical of a severe grade of pernicious anemia. There was an icteric tint to the skin and conjunctivæ. The general nutrition was excellent. There was a number of suspicious teeth and slight gingivitis. There was a soft systolic murmur at the cardiac apex.

During the first two weeks the patient ran an irregular temperature, reaching 100.5° F. almost every day, after which it remained normal with a few exceptions.

Laboratory.—Hb. (Sahli) = 10%. R. B. C. = 980,000. W. B. C. = 3,640. The stained smears showed a typical picture as shown by the chart. The urine showed a trace of albumin and some hyaline casts. The B. P. was 145/82. Her blood group was Group IV. An orthopœdic consultant reported slight pronation of the left foot, apparently the result of disuse. A proctoscopic examination revealed several large external hemorrhoids and a pedunculated polyp, which was removed. Following a dental examination five teeth were extracted and the sockets curetted. A gastro-intestinal series of X-rays were made with a normal report. The Wa. R. was negative.

TRANSFUSIONS

I. Four hundred and twenty-five cubic centimeters of matched whole blood. Lindemann method. There was a slight chill, and an elevation of temperature to 103.8° F. The patient was restless and nauseated. Donor, F.

II. Five hundred cubic centimeters of matched whole blood. Citrate method. A chill occurred during the operation, and elevation of temperature to 102° F. Donor, Ct.

III. Five hundred cubic centimeters of matched whole blood. Citrate method. There was no reaction except slight discomfort. Donor, Cl.

IV. Five hundred cubic centimeters of matched whole blood. Citrate method. There was a rather marked reaction with an elevation of temperature to 102° F., and the patient was generally uncomfortable. Donor, not stated.

V. Four hundred cubic centimeters of matched whole blood. Citrate method. No reaction. Donor, H. The urine showed a positive test for urobilin after this transfusion.

VI. Four hundred cubic centimeters of matched whole blood. Citrate method. No reaction. Donor, H. (same as for previous transfusion).

XII-20-17. Discharged.

Hb. = 70%. R. B. C. = 3,460,000.

SECOND ADMISSION

Medical No. 39120. Admitted January 4, 1918. *Diagnosis:* Pernicious anemia.

The patient returned to continue the treatment. She was in excellent condition. Hb. = 70%. R. B. C. = 3,460,000.

TRANSFUSIONS

VII. One thousand one hundred and fifty cubic centimeters of matched whole blood. Citrate method. No reaction. Donors, Cs. and Cl. (The bloods of the donors were matched against each other, as well as against the blood of the patient.)

VIII. One thousand cubic centimeters of matched whole blood. Citrate method. No reaction. Donors, A and Cr.

II-16-18. Discharged.

Hb. = 71%. R. B. C. = 3,500,000. W. B. C. = 6,100.

THIRD ADMISSION

Medical No. 39836. Admitted May 18, 1918. *Diagnosis:* Pernicious anemia.

Present Illness.—Since leaving the hospital, the patient had led a fairly active life. She had felt very well until one month before admission, when she began to notice that the least effort was tiring. There was slight vertigo on turning her head.

Hb. = 74%. R. B. C. = 3,000,000.

The *physical examination* was the same as upon previous admissions, but in addition there was slight edema of the ankles.

A gastric analysis showed a hydrochloric acid deficit of 7% and a total acidity of 5%.

TRANSFUSION

IX. Seven hundred cubic centimeters of matched whole blood. Citrate method. No reaction. Donor, Cs.

VI-19-18. The patient was discharged. Hb. = 85%. R. B. C. = 2,700,000. W. B. C. = 5,160.

FOURTH ADMISSION

Medical No. 40847. Admitted November 14, 1918. *Diagnosis:* Pernicious anemia. Oral sepsis. Fascicular myelitis.

The *complaint* was of numbness in both legs.

Present Illness.—About the first of August, 1918, the patient noticed numbness in the toes of her left foot, present night and day. This sensation later extended up the right foot and the calves of the legs, and the lower part of the abdomen did not feel natural. About two weeks after the onset of this complaint, the patient voided more frequently than usual and experienced some difficulty in holding her urine.

The *physical examination* was the same as upon previous admissions. In addition, the knee-kicks and ankle-jerks were hyperactive and the sense of touch was somewhat impaired over the lower extremities.

Hb. = 75%. R. B. C. = 3,056,000. W. B. C. = 11,800. The urine gave a negative test for urobilin. Following a dental consultation, one tooth was extracted, the socket curetted, and the gingivitis was treated.

TRANSFUSIONS

XI. Four hundred and fifty cubic centimeters of matched whole blood. Citrate method. No reaction. Donor, Hl.

XII. Five hundred cubic centimeters of matched whole blood. Citrate method. No reaction. Donor, Hs.

XI-26-18. Discharged. Vitally staining R. B. C. = 0.8%. Hb. = 100%. R. B. C. = 4,008,000. W. B. C. = 7,700.

FIFTH ADMISSION

Medical No. 42333. Admitted June 5, 1919. *Diagnosis:* Pernicious anemia. Achylia gastrica. Combined sclerosis.

From the time of discharge until six weeks before admission the patient remained in good condition. She maintained a hemoglobin of 90% or higher. About six weeks ago she began to feel weak and her Hb. was 80%. One week ago the hemoglobin examination was 70% and the R. B. C. count below three millions. There has been no improvement in the condition of her legs, the numbness extending a short distance above the knees.

The *physical examination* was the same as upon previous admissions.

Hb. = 45%. R. B. C. = 1,856,000. W. B. C. = 51,000. Vitally staining R. B. C. = 0.1%.

TRANSFUSIONS

XIII. Five hundred cubic centimeters of matched whole blood. Citrate method. During the transfusion an urticarial wheal was noted on the patient's left arm. About one hour later the patient became restless, vomited, and there was an elevation of temperature to 104° F. A specimen of urine voided seven hours later gave a positive test for Hb. No red blood cells were seen in the specimen. The temperature remained elevated on the following day, and she did not void, but had to be catheterized. On the following day (second), the patient was still quite drowsy. The skin and scleræ were more icteric, and numerous small petechiæ had appeared over the body. The urine showed no abnormal blood pigments. There developed marked herpes labialis, stomatitis and glossitis. Donor, L. E. W. Group IV.

VI-13-19. The patient was still mentally confused.

XIV. Four hundred and fifty cubic centimeters of matched whole blood. Citrate method. The patient began to feel chilly and to shake toward the end of the procedure, and the last 100 cubic centimeters of blood were not run in. The temperature rose to 101.4° F., but was down on the following morning. Donor, H. F. Group IV. Several other Group IV donors were tested at the same time. There was no agglutination with any of them, but in all except the donor used, there was hemolysis of the donor's cells by the patient's serum. This fact is interesting in view of the statement by Moss [6] that the serum of any one group will not agglutinate or hemolyze the corpuscles of other members of the same group, but will agglutinate and may hemolyze the corpuscles of any other group except those of Group IV.

VIII-28-19. The patient was discharged. Hb. = 85%. R. B. C. = 2,400,000. W. B. C. = 10,000.

Sixth Admission

Medical No. 43605. Admitted March 5, 1920. *Diagnosis:* Pernicious anemia. Combined sclerosis. Chronic cystitis. Achylia gastrica. Stokes-Adams syndrome. Obesity. The patient had felt fairly well since her last admission. She had had considerable difficulty in walking and only did so with support or the aid of a cane. She stood with a broad base and there was some tendency to swaying. This condition had been growing gradually worse. She had had electrical treatment with "violet rays" along her spine without improvement. She complained of a marked feeling of discomfort in both lower extremities, and there had been incontinence of urine at times. The last time the hemoglobin determination was made at her home it was reported as 80%.

The *physical examination* was the same as upon previous admissions. In addition, the knee-kicks and ankle-jerks were strongly hyperactive, and there was a sustained ankle clonus on the left, and a fairly well sustained clonus on the right. There was a patellar clonus on both sides. Plantar stimulation gave dorsal flexion on both sides. Muscle sense and appreciation of sharp and dull were impaired in both legs. Appreciation of hot and cold were somewhat diminished over both legs, as high as the knees. Appreciation for touch was diminished over the left leg and the left foot. The blood pressure was 126/76.

Laboratory. Hb. = 46%. R. B. C. = 1,430,000. W. B. C. = 13,050. Vital staining R. B. C. = 0.2%. The urine showed a faint trace of albumin, some hyaline and granular casts, and many W. B. C. in clumps. The urine gave a positive test for urobilin.

For ten days after admission the patient was kept on an experimental fat-free diet without any improvement in the blood picture.

TRANSFUSIONS

XV. Five hundred and fifty cubic centimeters of matched and grouped whole blood. Citrate method. The blood was run in very slowly. After receiving about 175 cubic centimeters of blood, the patient's respirations became somewhat sighing in character and then slightly dyspnœic. The transfusion was discontinued. The patient began to shake violently, but did not feel chilly. An hour later the temperature had risen to 104.6° F. She vomited and became somewhat irrational. She went to sleep at 8 P. M. and could not be roused during the night. On the following day the temperature remained elevated. The scleræ and skin were quite yellow. The patient was in a very low mental state; she seemed to be able to fix her attention, but did not respond to questions. She had not voided sixteen hours after the transfusion, and was catheterized for 350 cubic centimeters of reddish orange colored urine, containing many hyaline and granular casts and a few R. B. C. The urine gave a strongly positive test for hemoglobin with benzidine. Donor, Co. The blood of both the patient and the donor were regrouped; they were both Group IV. In rematching, on first trial there was a perfect match; on second trial, the recipient's serum *vs.* the donor's cells gave no agglutination or hemolysis; the donor's serum *vs.* recipient's cells gave no agglutination, but did give very slight hemolysis as shown by the presence of a few "shadows" under high power examination of the specimen. All incubations were carried on for at least one hour, usually longer.

III-21-20. The patient could be roused with difficulty and would respond to questions. There were marked herpes labialis and herpes zoster.

III-28-30. The patient had involuntary stools and involuntary voiding at times, but usually had to be catheterized. There was twitching of the upper extremities, and the stream of talk was occasionally incoherent and irrelevant. The pulse grew weak, but responded to strophanthin.

IV-2-20. The blood urea nitrogen was 74 mgm. per 100 cubic centimeters. The carbon dioxide of the plasma was 44.7 vol. %. The patient showed a constant temperature elevation as high as 102.8° F., and continued to require catheterization. Cystitis had become very marked and the urine showed a large amount of ropy pus in the specimen bottle. A urine culture showed a marked growth of *B. coli*. She began to have seizures, manifested by the loss of consciousness, and lasting from one to ten minutes. There was fixation of the eyes, twitching of the facial muscles, and coarse jerky movements of the hands and arms. Sometimes they began with hiccup, wretching, and vomiting of a light brown fluid. There was occasionally a very slow lateral nystagmus. During these attacks, the respiration became rapid and labored and there was foaming at the mouth. The radial pulse rate in these seizures was one half of the rate before or after the attack, and corresponded with the rate counted at the cardiac apex. For a period of a week the patient had numerous such seizures during the day and night, at the end of which time they ceased.

IV-4-20. Carbon dioxide of the alveolar air was 24 vol. %. A blood culture gave no growth.

XVI. As the patient had had such extremely severe reactions in previous transfusions, it was decided to attempt a transfusion of the serum of Group I, which contains no isohemolysins or isoagglutinins. A Group I donor was obtained and 500 cubic centimeters of blood was withdrawn in the usual method, and allowed to clot over night in the ice-box; the following day 235 cubic centimeters of the supernatant serum was pipetted off; centrifugalised at high speed to remove all cellular elements and inactivated at 56° C. for one hour. The serum was then diluted with 100 cubic centimeters of normal salt solution to render it less viscous. The diluted serum was injected intravenously in the usual method, by gravity. There was no reaction of any sort. Donor, O., Group I.

IV-18-20. The patient gradually became rational. The two small infected hypodermic areas which had followed the injection of strophanthin a week previously showed improvement. An early bedsore on the right buttock showed no improvement. There was

complete paralysis of both lower extremities, and complete loss of muscle sense, and of all sensations as high as the umbilicus.

IV-25-20. The patient's condition became very unsatisfactory. Her respirations became rapid and shallow with periods of hyperpnœa, and the pulse rapid, feeble and irregular. The temperature ranged from 101° (R) to 104.4° (R). The psyche was in a twilight state. There was a myocardial death at 8.10 P. M., with a temperature of 105° F. at death.

Analysis.—The patient in Case III had six admissions to the hospital in a little less than three years and during this period received about eight and one-half liters of blood in 15 transfusions, and 235 c. c. of blood serum in one transfusion. She suffered six reactions of varying degrees of severity, showing hemoglobinuria on two occasions. From the time at which she first came under observation until the development of a marked cystitis, and later, the transfusion of serum, the blood picture showed very little regenerative activity. Whether the evidences of bone-marrow stimulation, shown by the blood picture from April 2, 1920 until the time of death, resulted from the cystitis and hypodermic infections, or from the transfusion of Group I serum, it is impossible to say. However, the picture shows greater bone-marrow activity following the administration of blood serum. It is rather interesting that the hemoglobin and R. B. C. determinations showed a progressive improvement until the time of death. The acidosis shown by this case has been described by Barskey and Kahn[6] as one of the findings in pernicious anemia.

In endeavoring to explain these very severe reactions the first question which naturally presents itself, is: Were the donors' bloods really compatible with those of the recipients'? In all of the matchings carried out before transfusion the bloods apparently were compatible. In Case I, Transfusion V, the incubation of the preparations was carried on for only 30 minutes; hence we may reasonably assume that up to this time 30 minutes had been the usual incubation period in the routine blood matchings. Thereafter the preparations were allowed to incubate for one hour or longer. In Cases I and II the matchings were performed by the bacteriological laboratory; and in Case III all tests for compatibility were examined by at least two persons before being pronounced acceptable. In no instance were donors used when examination had shown either agglutination or hemolysis.

The blood groups of Cases I and II are not known. Case III was repeatedly found to belong to Group IV and only Group IV donors were used for transfusion of whole blood. This fact lends additional interest to the severity of the reactions experienced by Case III, as it has been stated by Drinker and Brittingham,[7] and reaffirmed by Lee[8] that the members of

CASE III

Date	Hb.	R. B. C.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. M.	L. M.	Tr.	Myelocytes	Anisocytosis	Poikilocytosis	Punctate basophilia	Diffuse basophilia	Nucleated R. B. C.	Macrocytes	Microcytes	Platelets	Vital staining R. B. C.	Transfusions
VII-21-17	10	980,000	3,640	..	..	..	..	..	..	..	+++	+++	+	+	0	....	....	Few.	..	7-23-17 425 c. c.
VII-27-17	27	1,448,000	4,200	..	..	..	..	..	..	..	....	....	..	..	..	....	....	....	..	7-30-17 500 c. c.
VIII- 8-17	38	1,688,000	5,000	..	..	..	..	..	..	..	....	....	..	..	..	....	....	....	..	8- 9-17 500 c. c.
VIII-19-17	41	1,808,000	2,400	..	..	..	..	..	..	..	....	....	..	..	..	....	....	....	..	8-21-17 500 c. c.
XI-26-17	68	2,248,000	7,480	56.0	6.0	.0	35.0	5.0	2.0	.0	++	++	..	..	0	....	....	Few.	..	12- 4-17 400 c. c.
XII-16-17	70	2,860,000	6,200	49.0	6.0	1.0	38.0	6.0	.0	.0	++	++	..	..	0	....	....	Few.	..	12-18-17 400 c. c.
XII-20-17	70	3,460,000	....	..	..	..	..	..	..	..	....	....	..	..	..	....	....	....	..	Second admission.
I-10-18	74	3,520,000	6,200	64.0	5.0	.0	26.0	5.0	.0	.0	++	+	0	0	0	+	....	Few.	..	1-21-18 1150 c. c.
II- 2-18	57	2,680,000	5,800	63.0	4.0	.0	25.0	7.0	1.0	.0	....	....	..	..	..	....	....	....	..	2- 5-18 1000 c.c.
II-16-18	71	3,500,000	6,100	..	..	..	..	..	..	..	....	....	..	..	..	....	....	....	..	Third admission.
V-18-18	70	2,736,000	4,600	..	..	..	..	..	..	..	+	+	..	..	..	....	....	... .	..	5-27-18 700 c. c.
V-29-18	82	2,720,000	4,800	..	..	..	..	..	..	..	+	+	..	..	-	....	....	....	..	6- 6-18 500 c. c.
VI-18-18	85	2,700,000	5,160	..	..	..	..	..	..	..	....	....	..	..	..	....	....	....	..	Fourth admission.
XI-14-18	75	3,056,000	11,800	53.2	2.8	.0	41.2	2.8	.0	.0	+	+	0	0	0	+	++	Normal.	..	11-16-18 450 c. c.
XI-19-18	80	3,528,000	6,800	62.0	4.0	.4	32.0	.8	.8	.0	+	+	0	0	0	....	....	Decr'd.	..	11-21-18 500 c. c.
XI-26-18	100	4,008,000	7,700	53.2	2.4	.8	?	3.6	.0	.0	0	0	0	0	0	....	....	Normal.	0.8	Fifth admission.
VI- 5-19	45	1,856,000	5,100	55.2	8.0	.0	36.4	.4	.0	.0	+++	+	0	+	1.0	++	....	Decr'd.	0.1	6- 7-19 500 c. c.
VI-15-19	43	1,472,000	4,700	37.6	6.0	.4	52.0	2.4	.0	1.6	++	++	0	0	0	....	....	Very few.	..	6-16-19 450 c. c.
VIII-28-19	85	2,400,000	10,000	..	..	..	..	..	..	..	....	....	..	..	..	....	....	....	..	Sixth admission.
III- 5-20	43	1,470,000	4,800	59.0	4.0	.0	36.0	1.0	.0	.0	+++	+++	0	+	0	+++	....	Very few.	0.2	3-19-20 175 c. c.
III-20-20	27	990,000	5,500	78.0	.0	.0	22.0	.0	.0	.0	++++	+++	0	+	0	....	....	None seen.	..	Cystitis developed.
IV- 2-20	26	770,000	17,650	90.0	.0	.0	10.0	.0	.0	.0	++++	+++	0	0	0	....	....	Decr'd.	..	....
IV- 7-20	20	790,000	15,500	83.0	1.0	.0	15.0	.0	1.0	.0	++++	+++	0	+	3	....	....	Decr'd.	..	4- 7-20 235 c. c. of Gr. I serum.
IV- 9-20	20	710,000	15,350	80.0	4.0	.0	11.0	3.0	2.0	.0	+++	+++	0	+++	19	....	....	Few.	..	... .
IV-24-20	46	1,430,000	13,050	83.0	.0	.0	13.5	2.0	.5	1.0	++	++	0	0	0	....	....	Few.	..	....

Group IV are universal donors, and may give blood for use with recipients of any other group. Lee states that donor and recipient need not be in the same blood group if the serum of the recipient does not agglutinate or hemolyze the R. B. C. of the donor, because the blood of the donor will make up only one-fifth to one-twelfth of the total blood volume of the recipient, and the donor's serum is thus diluted; moreover, the recipient's cells are protected by his own serum. Drinker and Brittingham have suggested that *in vitro* reactions are occasionally unreliable and Sydenstricker and others[1] state that it is safer to give recipients of other groups the washed R. B. C. of Group IV, than to attempt to use the whole blood. Case III proves conclusively that there are instances in which Group IV blood cannot be used with impunity, even in a member of the same group.

In an attempt to explain the mechanism of the reactions it is obvious that the reactions must have been due to either: (1) imperfect technic; (2) unavoidable factors in the procedure of transfusion; (3) or some undemonstrable incompatibility of the blood used.

There seems to be no reason for questioning the technic, as a uniform technic has been employed throughout the series, and a very large number of other cases have been transfused in the same manner without suffering serious reactions. Of necessity the Lindemann transfusions were always performed in the same manner. With the citrate method the apparatus offers one factor for discussion, since it has been shown by Stokes and Busman[9] that a certain type of new rubber tubing is responsible for some of the reactions to arsphenamin; they suggest that this may be a contributory factor to reactions with other intravenous methods. However, it is known that during the sixth admission of Case III the same apparatus, without change, had been used repeatedly on other cases without any reaction.

In an exhaustive study on the causes of reactions to citrate transfusions, Drinker and Brittingham[7] discuss factors which seem to be unavoidable in the present state of knowledge about transfusion. They conclude that reactions are due to: (1) Changes in the blood platelets, a part of the process of coagulation, (2) direct action of sodium citrate on R. B. C., rendering them more fragile and promoting hemolysis; (3) very rare gross incompatibilities which escape *in vitro* detection.

Coagulation has been considered a factor of importance by Novy and De Kruif,[10] who have shown experimentally with animals that blood becomes highly toxic in the precoagulation stage, and that the effects produced are those of anaphylatoxin, producing the same picture as anaphylactic shock. However this factor enters into every transfusion and as a rule we do not see severe reactions as a result of it.

In citrate transfusion the action of sodium citrate in rendering the red blood cells more fragile and thus promoting hemolysis may be a contributory factor. On the other hand the degree of hemolysis cannot be great, as Drinker and Brittingham do not mention having observed hemoglobinuria in any of their cases, while in the series under consideration, hemoglobinuria occurred four times, excluding transfusion V in Case I, in which the blood was later shown to have been incompatible. Moreover, it is far from proven that hemoglobin is as toxic as it is generally considered to be. Sellards and Minot,[11] in a study of the relationship between experimental hemoglobinuria and blood destruction in man, produced hemoglobinuria by the intravenous injection of the hemoglobin obtained from 4 c. c. to 33 c. c. of laked human R. B. C. They state: "One of the striking features of these injections is the absence of subjective symptoms after these comparatively large injections of hemoglobin. One normal patient received 28 c. c. of laked cells without any subjective symptoms and showed a slight hemoglobinuria. Another normal individual received 33 c. c. of laked cells. An hour after the injection a short mild chill developed which lasted about ten minutes, but it was not accompanied by nausea or headache. The patient became entirely comfortable after this chill, but during the first half of the night he did not sleep on account of flushing and a succession of hot and cold sensations. There was an intense hemoglobinuria and this discomfort continued for several hours after the active excretion of hemoglobin in the urine had ceased. The donor of cells for hemoglobin in this case was a member of Group IV." Twelve other cases showed varying degrees of hemoglobinuria without any subjective symptoms. They suggest that there are other factors in addition to the setting free of hemoglobin which play a rôle in the production of symptoms in those cases having some hemolysis following a transfusion. Likewise, Bayliss[12] has concluded from experimental injections of hemoglobin in cats that hemoglobin is non-toxic; he also found the stroma of the red blood cells to be non-toxic. In summarizing his results, he states, "It appears that the serious results of the transfusion of incompatible blood are not to be ascribed to the hemolysis as such, but are rather an aspect of the action of foreign serum protein analogous to that responsible for anaphylactic shock." Furthermore, Minot and Lee report three instances of reactions identical with those under consideration, but they do not state how many previous transfusions the patients had received. In one case they believe that the procedure hastened the patient's death, while in two cases it did not do so. They state, "In the case in which death was hastened the patient received in 12 days three transfusions of about 400 c. c. each. Following the first two there was no reaction, while following the third, from the same donor as was used for the first, the patient complained at once, particularly of headache; shortly vomiting occurred, the patient became toxic, the temperature rose, and marked jaundice developed, and he died in about 18 hours. No hemoglobinuria occurred. Another sick patient, shortly following a transfusion from a donor whom she had previously received blood from without ill effects, developed a similar severe reaction. The reaction consisted of a chill, temperature 102.5° F.; gastric symptoms and rapidly marked jaundice developed. Very slight hemoglobinuria occurred. Death occurred two weeks later. A similar, much less severe reaction occurred in a third very sick patient who died five days after the fourth transfusion in three weeks. The isoagglutination tests in these three cases showed no agglutination

or hemolysis and we verified them after the reactions in the first two cases. The donors and patients belonged to the same isoagglutination groups. Though these reactions may be of some sort of an isohemolytic nature, they are not of the known type. It would seem that they may have been due to the development by previous transfusions of some unknown and unrecognized antibody to the donor's blood." They further suggest that the reaction may have been due to a lowered tolerance to blood pigments which had accumulated during the blood destruction incident to the disease, and from the destroyed blood of previous transfusions, and that the subsequent introduction of more pigment had been sufficient to produce signs of toxicity.

The latter explanation does not seem so attractive as the former, since their argument is substantiated by the fact that the case which died had received the hemoglobin from 17 c. c. of laked R. B. C. in a previous experimental intravenous injection and had reacted with "marked toxic symptoms and an intense hemoglobinuria," whereas following the transfusion the reaction was more severe and there was no hemoglobinuria.

The statements of Novy and De Kruif, Sellards and Minot, Bayliss, and Minot and Lee thus have some bearing on the question of undemonstrable incompatibilities of blood producing severe reactions in transfusion. The manifestations of the reactions in the three cases under consideration are particularly suggestive of an anaphylactic reaction, as the picture resembles the immediate reaction occurring in "serum sickness" with dyspnœa, cyanosis and particularly urticaria. All three cases showed urticarial eruptions during the series of transfusions, and Case I, after one transfusion complained of puffiness about the eyes, although no œdema of the skin was noted. On another occasion the pains in the arms may have been joint pains. The tenability of the belief that the reaction is anaphylactic is further strengthened by the inability to demonstrate any incompatibility of the blood used, for the blood undoubtedly was incompatible in every instance in which a severe reaction occurred. This assumption would account for the absence of agglutination or hemolysis in the blood matchings, and we might expect to find a precipitin reaction between the incompatible bloods. It was considered desirable to study Case III from this viewpoint, but the opportunity did not present itself, as it would be necessary to have the donor's and recipient's blood before and after a transfusion in which a severe reaction had occurred.

A study of the foregoing cases emphasizes the importance of a few practical considerations, some of which have been stated before. The most significant fact is that there are certain cases of pernicious anemia in which it becomes increasingly difficult with successive transfusions to find compatible donors, and in which severe reactions will occur, no matter how carefully blood matching is carried out. In the present state of our knowledge of means for determining the compatibility of blood, transfusion is a self-limited process, and until more suitable methods are devised for blood matching no attempt should be made to transfuse these cases. The inadvisability of further attempts at transfusion should be indicated by the discovery that the patient reacts badly, particularly with hemoglobinuria, although no discrepancy is demonstrable upon rematching donor and recipient. As a matter of conjecture, it may be found by precipitation methods with the use of a hemolytic series that suitable donors are available for these cases, as precipitation tests are much more delicate than agglutination tests and might reveal a lesser degree of incompatibility. Furthermore, blood matchings should be carried out with the most extreme care and no preparation should be passed upon favorably in which the field does not show a perfectly uniform distribution. The period of incubation, except in emergencies, should be at least two hours, for Walter Baetjer[14] has seen some instances in which the bloods were slow agglutinators and produced no definite change in the preparation in one hour. The preparations should be examined for uniform distribution immediately after being set up. Whenever possible, the preparation should be allowed to incubate over night and be read on the following day. Whenever a recipient begins to show signs of reaction the transfusion should be discontinued at once; any remaining blood could be injected at the end of one or two hours if a severe reaction had not occurred. Pemberton[15] reports three deaths in which transfusion was completed after the beginning of a reaction. The development of an urticarial wheal during the course of a transfusion should be the sign for immediate cessation of the operation, and it seems doubtful whether the remainder of the blood should be used later.

Conclusions

1. In certain patients suffering from pernicious anemia, who have been transfused repeatedly, transfusion becomes self-limited because of the inadequacy of methods for selecting suitable donors.

2. This difficulty having once been discovered, no attempt should be made to transfuse these patients.

3. The severe reaction is probably due to an anaphylactic manifestation, and not to hemolysis *per se*.

4. Blood matching should be carried out with the greatest care; whenever possible, the incubation period should be at least two hours, or longer.

5. Blood serum free from cellular elements may produce bone-marrow stimulation.

6. Members of Group IV cannot be regarded absolutely as universal donors.

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THE FATE OF TRUE SOLUTIONS (PHENOLSULPHONEPHTHALEIN) AND COLLOIDS (TRYPAN BLUE) INJECTED INTO THE MAMMALIAN EMBRYO

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Savory, in 1857, was the first investigator to inject a substance into a living fetus and observe its appearance in the mother. He injected acetate of strychnine into two out of four fetuses in a dog. Nine minutes after the injection the mother exhibited tetany and died 28 minutes later. The two injected fetuses were dead; the uninjected fetuses were alive and showed no symptoms of tetany. He also injected two fetuses in a cat; 10 minutes later the mother showed signs of tetany and died in 17 minutes. The injected fetuses, although showing tetany, survived the mother; two uninjected fetuses showed no tetanic symptoms. He then injected six fetuses in a rabbit. The mother died in 18 minutes; the fetuses, which all showed signs of tetany, survived the mother for a short period. In another experiment, after strychnine had been injected into four fetuses in a dog, the animal showed tetanic spasms after 30 minutes. Savory noted that in nearly all his experiments the fetuses were less susceptible to strychnine than the mother.

Savory's interesting observations were confirmed by Gusserow (in 1878) who injected a series of 24 pregnant rabbits, 7 dogs, and 5 cats with strychnine. His results were very similar to Savory's. He noted that the more advanced the gestation, the more rapidly strychnine passed from fetus to mother. Strychnine convulsions appeared in the mother in from 11 to 36 minutes after the injections, and usually resulted in death in from 30 to 45 minutes. Gusserow concluded, from Savory's experiments and from his own, that substances can pass from fetus to mother.

Preyer (in 1885) observed the passage of several other substances from fetus to mother. He injected 0.2 c. c. of a 12 per cent solution of hydrocyanic acid into a mature fetal guinea-pig; two minutes later the mother had convulsions, became dyspnœic and died after four minutes. The injected fetus was dead; and uninjected one was still alive at the time of death of the mother. In another similar experiment the mother exhibited convulsions one and one-half minutes after the injection of 0.2 c. c. of a 12 per cent solution of hydrocyanic acid. Preyer also experimented with nicotine. This drug, when injected into several guinea-pig fetuses, produced clonic convulsions in the fetuses in one and a quarter minutes, but in the mother only mild symptoms of poisoning, which did not result in death.

In another experiment a quantity of curarine, sufficient to kill an adult guinea-pig in 15 minutes, was injected into a guinea-pig fetus. The mother became completely paralysed and died in 80 minutes. The injected fetus, as well as two uninjected ones, showed no symptoms of poisoning. Several other experiments with curarine gave similar results.

Lannois and Briau (in 1898) investigated the passage of several other substances from fetus to mother. They injected sodium salicylate into guinea-pig and rabbit fetuses, and in three instances found the drug excreted in the urine of the mother at the end of one hour. With potassium iodide three trials were negative, but in one instance they were able to detect the substance in the maternal blood stream at the end of an hour. With methylene blue they succeeded in recovering the substance from the maternal urine in five out of six attempts. At the end of two hours only chromogen was obtained from the urine, but in from six to seven hours the unchanged dye-stuff was finally excreted. They too observed that the passage of substances from fetus to mother was most rapid near term.

Baron and Castaigne (in 1898) found that potassium iodide, injected into guinea-pig fetuses, appeared in the maternal urine in 40 minutes. When injected early in pregnancy they noted the passage of the same drug in 30 minutes. Methylene

blue injected underneath the scalp of a human fetus during labor appeared in the maternal urine in one-half hour. They found that the substances do not appear in the urine if the fetuses are dead.

Guinard and Hochwelker (1899) observed the passage of rosanilin from fetus to mother in four instances, the shortest interval required for the drug to reach the maternal urine being 25 minutes. They found that death of the fetus invariably suspends the fetal-maternal passage of a substance. They injected strichnine and aconite repeatedly into dead fetuses without any effect upon the mother.

Charrin (1898) injected diphtheria toxin into fetuses and observed symptoms of intoxication from it in the mother.

Kreidl and Mandl (1903) injected atropine, adrenalin, pilocarpine, physostigmine, and phloridzin into a number of rabbit, cat, dog, goat and monkey fetuses. Atropine (1 c. c. of a 1 per cent solution) caused dilatation of the pupils in the mother, commencing 10 minutes after the drug had been injected into the fetuses. Pilocarpine (several cubic centimeters of a 1 per cent solution) caused extreme salivation in the mother within 10 minutes after its injection. The passage of physostigmine was demonstrated by injecting it into the fetuses of an animal which had been previously curarinized. On the injection of physostigmine the sciatic nerve resumed its irritability toward electric stimuli. Finally, phloridzin was shown to cause melituria in the mother within three hours following the injection of several cubic centimeters of a 5 per cent solution into the fetuses. Adrenalin was the only drug from which an effect on the mother could not be demonstrated. Numerous experiments with adrenalin led to the conclusion that it decomposes and loses its pharmacological activity before it traverses the placenta. Kreidl and Mandl mentioned no differences of time required by the drugs to pass from fetus to mother in the various species of animals employed by them.

In addition to the observations upon the fetal-maternal passage of substances which have been injected directly into the fetuses, several experimenters have investigated the possibility of substances passing from the amniotic fluid into the maternal blood stream. Thus Gusserow (1858) injected strychnine into the amniotic sacs of a series of rabbit, cat, and dog fetuses. In three of these experiments the mother showed symptoms of strychnine poisoning in 15 to 20 minutes. In seven others strychnine poisoning failed to develop in the mother. Preyer (1885), analyzing Gusserow's work, came to the conclusion that the three positive experiments afforded excellent proof of the passage of substances from the amniotic fluid into the maternal blood stream. In the seven negative experiments he was able to point to some technical error, the use of chloroform, or the death of the fetus, as an explanation for the failure of the strychnine to produce poisoning in the mother.

Bar (1881) undertook two similar experiments. He injected 20 drops of a strychnine solution into the amniotic sac of a rabbit fetus. After 20 minutes the mother had convulsions. He introduced a similar amount into the amniotic sac of a fetus of another animal; in 20 minutes the mother had severe convulsions.

Toerngren (1888) injected iodide of potassium in doses of one or two grams into the amniotic sacs of rabbits. The substance later appeared in the urine of the mother. The time required was at least 45 minutes, and passage occurred whether the fetuses were at term or in the middle of development.

In addition to the question of transmission of substances injected into the fetus through the placenta, the possibility of excretion by the Wolffian body and the metanephros during fetal life have been investigated experimentally. Thus, Wiener (1881) injected sodium indigo sulphonate through the uterine wall into the subcutaneous tissue of several dog fetuses. In 25 minutes the substance appeared in the convoluted tubules of the kidneys, and a few minutes later in the fetal bladders. The substance similarly injected into rabbit fetuses appeared in 20 minutes in the convoluted tubules, and subsequently in the fetal bladders. The glomeruli remained uncolored. Wiener also injected diluted glycerine subcutaneously into rabbit fetuses, whereupon hemoglobinuria resulted in one and a half hours. He also injected a solution of potassium ferrocyanide into the amniotic sac of a rabbit and after two and a half hours detected it in the fetal urine.

Bar (1881) repeated Wiener's experiments, using potassium ferrocyanide. At the end of 40 minutes several drops of urine in the bladder gave the Prussian blue reaction.

Bakounine (1895) injected indigo carmine into the omphalomesenteric vessels or the aorta of developing chicks, and observed the excretion of the coloring matter by the tubules of the Wolffian body. The glomeruli never exhibited the dye.

Recently Firket (1920) has reported the results of injecting a solution of sodium ferrocyanide and iron ammonium citrate into the umbilical vein of cat fetuses. On sacrificing the mothers at intervals varying from 15 minutes to several hours and examining the fetuses, Prussian blue was observed in the convoluted tubules, urine, and allantoic fluid.

Mention must also be made of the experiments of Krukenberg (1885) on rabbits. After the injection of potassium iodide into the maternal blood stream he found traces of the substance in the fetal kidneys.

In the present experiments the fate of phenolsulphonephthalein and trypan blue injected into fetuses was investigated. Phenolsulphonephthalein was selected because it is a crystalloid, diffuses readily and is non-toxic. Trypan blue was used because its forms a colloidal solution, is relatively non-toxic and its behavior in the adult organism is well understood.

Phenolsulphonephthalein

Phenolsulphonephthalein was injected into a series of cat and guinea-pig fetuses by the following technique: The pregnant animal was anæsthetized with ether, a laparotomy performed, the bladder exposed, the urethra ligated, and a bent glass cannula tied into the bladder. The cannula was allowed to drain into a beaker containing a 1 per cent solution of sodium hydroxide. The uterus was next exposed and a fetus palpated. A syringe containing sterile phenolsulphonephthalein solution and bearing a 16-gauge needle was inserted

through a nick in the uterine wall, through the fetal membranes into the peritoneal cavity of the fetus. One cubic centimeter of phenolsulphonephthalein was injected into the peritoneal cavity of the fetus and the syringe withdrawn. The uterus was carefully replaced in its normal position and the laparotomy opening closed. The animal was kept under light anæsthesia. The interval elapsing between the time of injection and the first appearance of the dye in the maternal urine was noted; as soon as this occurred the animal was sacrificed. The fetuses were removed from the uterus, and the bladder content of the injected fetus was tested for phenolsulphonephthalein. The urine of the adjacent fetuses and fluid contained in their membranes were also tested for the presence of the dye. The results of these experiments are presented in Table I.

It will be seen that phenolsulphonephthalein, when injected into the peritoneal cavity of the fetus, is absorbed by the fetal blood-stream and conveyed to the placenta, through which it slowly diffuses into the maternal circulation. The time required for the drug to appear in the mother is quite variable for individuals of the same species. No difference is observed between the cat and guinea-pig in the time required for the passage of the dye from fetus to mother. Further, it is seen that phenolsulphonephthalein is readily excreted by the fetal kidneys, for it was found in the fetal bladder in every instance in which sufficient urine could be collected to test for its presence. None of the drug could be detected in the urine or amniotic fluid of the adjacent uninjected fetuses.

Trypan Blue

Trypan blue (1 c. c. of a 0.5 per cent solution) was injected into the peritoneal cavities of cat and guinea-pig fetuses by a similar technique. Table II shows the results of these experiments.

It will be observed that trypan blue could not be detected in the maternal urine during the period of 5 hours while the animals were under observation. At the termination of the experiment the dye in every instance was found in the fetal kidneys. No trypan blue was observed in the uninjected fetuses.

In a second set of experiments trypan blue was injected into the peritoneal cavities of the fetuses under aseptic precautions. After closing the abdomen of the pregnant animal it was allowed to recover from anæsthesia. The animals were finally sacrificed at intervals of 24, 48 and 72 hours, respectively. In none of these cases was trypan blue found at any time in the maternal urine.

In every instance in which the mother was allowed to survive 24 hours or longer, the fetus which had received an injection of trypan blue was found vitally stained. In the gross the staining was quite striking. The placenta and fetal mem-

TABLE I

Animal	Crown-rump length of injected fetus	Substance and quantity injected	Where injected	Time of appearance in maternal urine	Urine of injected fetus	Urine and amniotic fluid of uninjected fetuses	Condition of injected fetus at time of removal from uterus
Guinea-pig 1...	95 mm.	1 c. c. phenolsulphonephthalein.	Intraperitoneally.	1 hr. 45 minutes.	Positive.	Negative.	Living.
Guinea-pig 2...	112 mm.	1 c. c. phenolsulphonephthalein.	Intraperitoneally.	55 minutes.	Undetermined.	Negative.	Living.
Guinea-pig 3...	67 mm.	1 c. c. phenolsulphonephthalein.	Intraperitoneally.	1 hr.	Positive.	Negative.	Living.
Guinea-pig 4...	118 mm.	1 c. c. phenolsulphonephthalein.	Intraperitoneally.	40 minutes.	Positive.	Negative.	Living.
Guinea-pig 5...	106 mm.	1 c. c. phenolsulphonephthalein.	Intraperitoneally.	55 minutes.	Positive.	Negative.	Living.
Cat 1..........	105 mm.	1 c. c. phenolsulphonephthalein.	Intraperitoneally.	1 hr.	Positive.	Negative.	Living.
Cat 2..........	116 mm.	1 c. c. phenolsulphonephthalein.	Intraperitoneally.	2 hrs. 25 minutes.	Positive.	Negative.	Living.
Cat 3..........	125 mm.	1 c. c. phenolsulphonephthalein.	Intraperitoneally.	2 hrs. 15 minutes.	Undetermined.	Negative.	Living.
Cat 4..........	115 mm.	1 c. c. phenolsulphonephthalein.	Intraperitoneally.	45 minutes.	Positive.	Negative.	Living.
Cat 5..........	87 mm.	1 c. c. phenolsulphonephthalein.	Intraperitoneally.	1 hr. 35 minutes.	Positive.	Negative.	Living.

TABLE II

Animal	Crown-rump length of injected fetus	Substance and quantity injected	Where injected	Time of appearance in maternal urine	Urine of injected fetus	Urine and amniotic fluid of uninjected fetuses	Condition of injected fetus at time of removal from uterus
Guinea-pig 1...	92 mm.	1 c.c. 0.5% trypan blue.	Intraperitoneally.	None in 5 hours.	Positive.	Negative.	Living.
Guinea-pig 2...	122 mm.	1 c.c. 0.5% trypan blue.	Intraperitoneally.	None in 5 hours.	Positive.	Negative.	Living.
Cat 1..........	75 mm.	1 c.c. 0.5% trypan blue.	Intraperitoneally.	None in 5 hours.	Positive.	Negative.	Living.
Cat 2..........	32 mm.	1 c.c. 0.5% trypan blue.	Intraperitoneally.	None in 5 hours.	Positive.	Negative.	Living.

branes of the injected fetus were dark blue, while the uterine musculature and decidua in the region of the injected fetus were unstained. No staining of the placentæ or membranes of the neighboring uninjected fetuses was observed. On rupturing the membranes of the injected fetus the amniotic fluid was observed to be quite blue. In the cat fetuses an additional blue coloration was noted in the allantoic fluid. The umbilical cord, as well as the fetus itself, appeared deep blue. On gross sagittal section of the fetus its tissues were observed to be uniformly blue with the exception of the brain and cord, which were unstained.

A detailed description of the microscopic appearances of these vitally stained fetuses will be reserved for a future paper, but the main features of the staining will be here briefly outlined. Trypan blue occurs in many of the tissues of the fetus in the form of characteristic cytoplasmic granules. It occurs most abundantly in the liver and kidneys. The Kupffer cells of the liver contain numerous tiny, brilliant blue granules within their cytoplasm. The epithelial cells of the convoluted tubules of the kidneys are heavily laden with blue pigment. Trypan blue has not been deposited elsewhere than in the convoluted tubules, for the glomeruli and collecting tubules are unstained. So-called clasmatocytes, pigmented with trypan blue, occur throughout the loose connective tissue of the fetus. The cells constituting the delicate stroma of the umbilical cord and fetal membranes contain minute granules of dye within their cytoplasm. The amniotic epithelium contains similar cytoplasmic granules. In the placenta the dye occurs in the form of tiny granules in the cytoplasm of the cells which form the delicate stroma supporting the fetal vessels. It is also observed in the endothelial cells of the placental capillaries. Dye is not seen in the chorionic epithelium or in any of the maternal elements of the placenta.

The liver and kidneys of the mother were examined microscopically for trypan blue, but no trace of the dye could be discovered.

Summary

1. Phenolsulphonephthalein, which represents an easily diffusible, non-toxic dye, is absorbed from the peritoneal cavity of the fetus. It passes slowly from the fetal blood stream through the placenta into the maternal blood stream and is excreted by the maternal kidneys. It is also excreted by the fetal kidneys, since it is found in the fetal urine. This observation demonstrates that the fetal kidneys are capable of excretion, but sheds no light on the extent to which they act as a pathway of excretion before birth. No difference was noted between the cat and the guinea-pig as to the time of transmission of the dye by the placenta, but a marked difference in the time required was observed in individuals of the same species.

2. Trypan blue, a less diffusible, non-toxic colloidal dye, is also absorbed from the peritoneal cavity of the fetus. It is not excreted in the maternal urine, and microscopic examination shows that it is not transmitted by the placenta to the maternal organism. It is excreted by the fetal kidneys. The observations with trypan blue show that the placenta is incapable of transmitting an inert foreign colloid from fetus to mother, and hence it is probable that the placenta does not transmit the colloidal products of fetal excretion unless they are first converted into simpler, readily diffusible, substances.

3. The fetus becomes vitally stained after injections of trypan blue into the fetal peritoneal cavity. The dye is stored in the endothelial cells of the liver, and also accumulates in the ephithelial cells of the renal convoluted tubules. The dye is absorbed by clasmatocytes which occur throughout the fetus and in the placenta and fetal membranes.

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PROCEEDINGS OF SOCIETIES

THE JOHNS HOPKINS HOSPITAL MEDICAL SOCIETY

DECEMBER 6, 1920

1. Drainage of the Common Bile-Duct through the Cystic Duct. Cystico-choledochostomy. (Abstract.) DR. MONT R. REID.

Twelve months ago Dr. Halsted and I made a brief report to this Society on the subject of drainage of the common bile-duct. Up to that time the ductus choledochus had in four instances been drained in the manner about to be described, two of the patients being exhibited at the meeting. In a letter to the editors of the *Journal of the American Medical Association* (published December 20, 1919, vol. 73, pp. 1896 and 1897) Dr. Halsted advocated the careful and complete closure of the incision into the ductus choledochus and the drainage of this duct by a tube passed well into it by way of the ductus cysticus. For this procedure he proposes the term cystico-choledochostomy, aware, of course, that the choledochotomy is not embraced in it.

In our older hospital records we find that in a few instances a tube has been passed from the cystic into the common duct, but only in cases in which the common duct had not been incised. For example, in 1899, Dr. Halsted being able to remove stones from the common bile-duct by forcing them back through the cystic duct, drained the former by a tube passed through the latter. Similarly, Sowers in 1904 drained the common duct through the short stump of a friable cystic duct. Finney and Follis, also for the same obvious reason, drained the common duct in this manner in 1903 and 1904. In none of these cases, however, had the common duct been incised.

On March 24, 1917, Dr. Halsted first employed the method which we now advocate for all cases in which there is no definite contraindication. The patient, a woman 56 years old, was intensely jaundiced. A shrunken, chronically inflamed gall-bladder containing stones was excised, and several large soft stones were removed through an incision into the greatly dilated common duct. A catheter, No. 19, French, was passed through the cystic into the common duct and retained by a suture of catgut, No. 00. The long incision in the common duct was carefully closed with two rows of interrupted chromic catgut (No. 0) sutures. About this tube four small drains were placed. The patient's convalescence was remarkably rapid. All of the drained bile came by way of the tube and thus could be accurately measured. On the fourth day the tube was clamped for 11 hours; on the fifth for a longer period. As the clamping caused the patient no distress and as there was no peritubal leakage, the tube was removed on the sixth day. Except for a faint stain on the first dressing there was no evidence thereafter of even a drop of bile in the sinus tract. The wound healed promptly and the patient walked out from the hospital three weeks after admission. Thus, for the first time in this clinic, drainage of a sutured choledochus was maintained by a tube passed into it through the ductus cysticus. In the previous cases of cystico-choledochostomy the common duct, as stated above, was not incised, the stones having been removed through the cystic duct.

Although the impression made by this experience was a profound one, it was not until Dr. Halsted, two and one-half years later, had been operated upon for stones in the common duct and experienced the distressing consequences of the loss of all the bile for a period of three weeks, that he insisted upon the closure of the incision into the choledochus and the drainage of this duct by way of the cysticus, whenever possible. He had indeed requested that this procedure be adopted in his case, but as the cystic duct entered the common duct from behind, it was thought better, being simpler, to drain the choledochus in the orthodox fashion—in the way recommended and practised by surgeons the world over. On the second day bile began to leak about the tube and after the fourth day the tube (removed the fifth day) conveyed none of the bile which for three weeks poured in great quantities from the sinus. Food was positively repulsive, and the sense of taste was so far lost that frequently he seemed unable to distinguish one kind of food, meat or vegetable, from another, except by sight. By the 14th day emaciation and weakness were so marked that grave concern for his life was felt. In three weeks he lost 30 pounds in weight.

The operation is performed as follows: The cystic and common ducts being well exposed, the cystic artery ligated, the cystic duct clamped and divided about 2 cm. from its origin, the gall-bladder excised, the common duct incised and all discoverable stones removed, a probe, as large as possible, is passed into the duodenum. The stump of the cystic duct is then investigated and, if necessary, stretched with a clamp or other instrument. It is my preference to introduce the tube of the selected size through the cystic into the common duct before suturing the incision into the latter. The tube being properly located is maintained in place by one stitch of catgut No. 00, passed through its side and into the wall of the cystic duct. The incision into the choledochus is closed by one or, preferably, two rows of interrupted fine silk (No. 0) sutures. On several occasions I have used the finest catgut (interrupted sutures) for the inner row. One should test the line of suture by injecting salt solution through the tube. The suture line may then be protected from the drains by overlaid fat or other tissue. Three or four very thin Halsted drains or slender cigarette drains are placed about the rubber catheter. The cystic duct should be made to hug the catheter closely. It may ultimately seem advisable to employ a tube larger than those that we have been accustomed to use, but we must bear in mind the fact that the chief function of the tube is to relieve tension on the line of sutures of the common duct until union of this wound is firm. The employment of the largest possible size of tube in the hope that perhaps a stone may escape through it is inadvisable. Although small concretions have occasionally been known to pass by way of a tube, it is more than probable that, when this has occurred, others were left to find

their way into the duodenum by the natural route. The drains are removed, some on the second or third day, the remainder on the third, fourth, or fifth day. The catheter in the duct should not be disturbed until there is reason to believe that the wound in the common duct is firmly healed. In one instance it was left, as an experiment, for three weeks. Observations of various kinds were made during this period. On removal of the tube the bile, except for a few cubic centimeters, passed by the natural route.

When the cystic duct is obliterated, one should make, if feasible, a second opening into the choledochus, just large enough to admit a small tube, and completely close the original incision. I tested this in one case at Dr. Halsted's suggestion, and with satisfactory result. The outflow of bile ceased promptly on withdrawal of the tube. In all of our cases the tube has been directed towards Vater's diverticulum and in none of these did it become kinked or obstructed.

SUMMARY

1. Prolonged leakage of bile is distressing in its consequences to those who withstand it and has occasionally, both directly and indirectly, been responsible for the death of the patient.

2. The incision into the common duct should be closed and the tension on the suture line be relieved by a tube passed through the cystic duct.

3. This tube, if properly fitted, may function for weeks, conveying the bile, without peritubal leakage, to the surface.

4. On removal of the tube the discharge of bile through the fistula ceases either immediately or within a day or two, provided the common duct is not obstructed.

5. The patency of the duct may and should be tested by clamping the tube.

6. Healing of the abdominal wound is less likely to be delayed if uncomplicated by the leakage of bile—bile which is quite invariably infected.

7. Following removal of the cystico-choledochostomy tube there has been no leakage of bile in about half the cases, and no patient has drained externally a significant amount of bile for more than two days.

2. An End-to-End Anastomosis of the Large Intestine by Abutting Closed Ends and Puncturing the Double Diaphragm with an Instrument Introduced per Rectum. Dr. W. S. Halsted.

My interest in end-to-end suture (circular enterorrhaphy) of the intestine has had its exuviation periods. The impulse for the current experimental study was given by experiences in the case of a friend upon whom in the course of a very difficult and quite desperately serious operation for uterine and ovarian neoplasms it became necessary to excise a portion of the sigmoid flexure of the colon and to perform within the pelvis an end-to-end suture of this bowel. The operation, according to the testimony of competent observers, was performed in a masterly manner, the competent surgeon having the secure foundation that experimental work in the laboratory alone can give. A fecal fistula through which escaped all of the intestinal contents formed at the line of the circular suture, which presumably broke down more or less completely. For five weeks or more the patient had rigors and high fever, and when her life was almost despaired of the entire picture changed spontaneously within an hour or two and a rapid and uninterrupted convalescence followed. The operator was impressed with the filthiness of the methods of performing end-to-end anastomosis of the colon, particularly in this situation. The immediate incentive for again taking up the subject of intestinal suture was, as I have said, the outcome of weeks of anxious observation of this stormy convalescence.

The allotted time permits only the briefest reference to salient facts in the history of intestinal suture, and I shall confine myself to the consideration of advances which to me seem modern, ancient though they may appear to those of you born years after this hospital was opened.

In the autumn of 1886, in the laboratory of Dr. Welch and with the assistance of Dr. Mall, I undertook the study of intestinal suture. Surely no one ever worked under happier auspices or with more stimulating companions. A few years later Senn was experimenting with his plates of cartilage, and then Abbe with catgut rings. The fact that such contrivances could have been seriously advocated by representative surgeons registers the crudity of intestinal surgery in our country about 30 years ago.

Our experiments were conducted almost daily for about six months. What they yielded is recorded in the papers of Mall and myself. Pertinent to our present study is the fact that the importance of the submucosa was recognized. I have only recently discovered that Gross had mentioned this coat in 1843, but in the intervening years its very existence was altogether overlooked, and every surgeon believed that it was possible to take and advocated the taking of a peritoneal stitch for the final row. The necessity for including in each stitch at least a part of the submucous coat being now recognized, surgeons have concentrated their attention on the devising of a suture which should be as nearly as possible bacteriologically clean. Numerous instruments and methods designed to lessen the amount of contamination have been contrived but with so little success that the end-to-end suture is quite universally performed to-day essentially as it was by Czerny in 1878 or by myself in 1886, or by Connell in 1892. The Murphy button (December, 1892) will in my opinion soon be obsolete. The several objections to its use which at the outset were offered have proved valid, and I would add the obvious one, that ideal healing should not be expected to take place on the confines of sphacelated tissues. Nevertheless this ingenious contrivance has enjoyed a marvellous endorsement both in this country and abroad; it tempted incompetent surgeons to essay operations for which they were unequipped, and made appeal to the operator who overestimated the value of time—of the time saved to the patient and lost to himself. Senn in his comprehensive and valuable paper on intestinal suture calls attention to the interesting fact that an Argentine surgeon was awarded a gold medal by the Peruvian Government for his invention of a button which in principle is essentially the

same as Murphy's: "A few days ago I received an interesting brochure from Adelbert Ramaugé, professor of surgery in the medical faculty of Buenos Ayres, entitled 'Enteroplexie,' a paper which he read at a meeting of the International Medical Congress of South America, January 20, 1893, and which received the first prize, a gold medal, from the Peruvian government. In this paper I find the description of an instrument which is intended for the same purpose as the Murphy button and which bears a strong resemblance to it."

As the bulkhead suture of Dr. Gatch and myself did not prove to be strictly an aseptic one, I finally abandoned attempts to perform it. But it taught us and Dr. Grey, who simplified it, that a great amount of intestine could safely be turned in—an amount greater than is inverted by the procedure about to be described. The remarkable results obtained by Gatch, with a method which he subsequently developed, deserve wider recognition and furnish convincing confirmation of the above statement in regard to the depth of the flange which may be turned in without fear of causing obstruction.

The current experiments of Dr. Holman and myself, although few, are sufficient in number to have demonstrated the feasibility of the idea, which was to abut and sew together the aseptically closed ends of the intestine and trust to the rapid disintegration of the occluding purse-string of fine catgut for the reestablishment of the bowel's lumen. If advisable, a colostomy would be made proximal to the anastomosis. Dr. Bloodgood tells me that his best results in resection of the large intestine for carcinoma have been obtained in the patients who on admission were so ill that only a colostomy could be ventured, and in those already provided with a preternatural anus; and Dr. J. Shelton Horsley in his paper on "Resection of the Cæcum and Ascending Colon" has said enough to indicate an inclination on his part to advocate the use of a protective colostomy. Our procedure is as follows: The muscular coats of the intestine are stripped from the submucosa for about 2 cm. towards the piece to be resected; finely basted, purse-string sutures of catgut are taken in the submucosa, and the bowel divided with a cautery knife between them at the sites of election; then, with the finest point of a Paquelin, the centers of the stumps to be approximated are cautiously burnt; and now the closed, abutted ends of the intestine are united by mattress sutures.

The first five sutures, alternately green and black if one chooses, are used as stays between which, on the stretch, the supplementary ones are taken. Two of the stays are placed very close together, one on each side of the mesenteric attachment; the third is taken at the free border of the gut; the fourth and fifth, one on each side, midway between the two borders. None of the stay sutures is tied until all of these have been placed. Each intervening stitch is tied when made. The nearer the line of suture to the stumps the less, of course, the amount of inturn, but the operator should not let the fear of inverting too much deter him from providing for the apposition of sufficiently broad peritoneal surfaces. On the other hand, the diaphragms had better not be flappish, although a little slack is permissible.

After the above report was made it occurred to me that one might easily develop a method for puncturing the double intestinal diaphragm from below. A short cylinder of wood containing four housed knives is introduced *per rectum* by an assistant against whose manœuvres the operating field is of course protected. This cylinder should approximately fill the bowel in order to center the knife perfectly. Inside the gut it is picked up at the brim of the pelvis by the operator and pressed on to the desired point. A second short cylinder, a trailer, threaded on a flexible guide, follows the first, to enable the outside assistant to push the latter to within reach of the operator's hand. The apparatus may be slipped along to any part of the large intestine. Precise details of the apparatus will be given in a subsequent communication.

Dr. Holman will now tell of our results and exhibit the specimens.

3. Exhibition of Specimens Obtained in End-to-End Anastomoses Performed According to the Method Described by Dr. Halsted. Dr. Emile Holman.

The three specimens presented illustrate the results obtained from the form of suture under discussion to-night.

In dog No. 1, the anastomosis was performed in the descending colon six inches above the anus in the manner described by Dr. Halsted. Through a second incision on the right, the appendix was delivered into the wound and sutured in place, but was not opened until the following day. During the next few days the dog was obviously ill, but without vomiting or distention. On the fourth day, the dog ate some meat and on the following day the first bowel movement since operation occurred. There was an immediate change for the better in his condition, and the dog seemed perfectly well on the seventh day, when he was sacrificed and the specimen obtained. There was no peritonitis or free fluid in the peritoneal cavity. The line of union was well healed and only a few filmy adhesions of the omentum were present around the line of anastomosis. A well defined opening was present, 4.5 cm. in circumference, which easily admitted the little finger. As you may see in the specimen, the mucosa is well approximated and healing is well advanced.

The second specimen was obtained from the descending colon on the nineteenth day after the anastomosis. In this case no enterostomy or appendicostomy was performed, and although slight vomiting occurred on the night of the operation, probably from the effects of ether, there was no further vomiting or distention. The dog partook of meat on the third day, and the first bowel movement occurred during the third night following the operation. The specimen shows an opening 1 cm. in diameter, with excellent healing and approximation of the mucosa. It should be noted that this specimen was obtained by resection of the bowel according to Dr. Halsted's method, and the dog is alive and happy to-day, January 27.

The third specimen is particularly interesting in that the same method was employed in the small bowel about two feet beyond the duodenum, without an enterostomy. The specimen was obtained 21 days after the first operation by resection of

the bowel according to Dr. Halsted's method; this dog recovered from the second operation and is well to-day. No distention or vomiting occurred at any time, food was taken on the fifth day, and a bowel movement was noted on the sixth day.

In addition to these successful cases, mention should also be made of the death of three dogs upon whom the method was employed, without a safeguarding enterostomy. In each case, the catgut sutures surrounding the bowel ends failed to disintegrate and give way. As a result distention developed proximal to the anastomosis, leakage occurred at the line of union, and peritonitis followed. We have been using No. 0, 00, and 000 plain catgut for the purse-string suture. We might hope for uniform success if a catgut could be obtained which would, without fail, become disintegrated within 12 hours.[1]

[1] Since making the above report we have in three instances used raw unsterilized catgut. Two of the dogs had bowel movements within 24 hours and recovered without apparent discomfort; but the third dog died from obstruction about 36 hours after the operation. The line of suture, however, seemed to be firm and there was no peritonitis.

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CONTENTS

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THE DANGERS TO LIFE OF SEVERE INVOLVEMENT OF THE THORAX IN RICKETS

By Edwards A. Park and John Howland

The thorax is affected like all other parts of the skeleton in the course of severe and prolonged rickets, and frequently becomes the seat of most extensive deformities. A mild degree of involvement of the thorax in the rachitic process is itself of little or no importance. More severe involvements, however, constitute a veritable menace to life itself. The object of this paper is to call attention to a little recognized but very definite symptom-complex which is accompanied by characteristic autopsy findings. The symptoms and pathological changes in question are the direct result of the diseased condition of the thorax. We also wish to discuss the mechanism by which these symptoms and pathological changes are produced and life is threatened.

The material, which forms the basis for our study, consists of 32 children between the ages of 8 months and 4½ years. All the children exhibited well-marked rachitic deformities of the thorax. In the case of six of them, however, the rachitis had not progressed far enough to produce great weakening of the thoracic wall and in four there had occurred healing or at least thickening of the ribs to an extent sufficient to make the deformed thorax rigid. Excluding these ten cases, which served only for the study of the deformities of the thorax, there remain 22 cases. These 22 cases form the actual basis for this report. In all of them there were present not only the characteristic deformities of the thorax but also the essential element, great weakening of the thoracic wall, and in all there were the characteristic train of symptoms and X-ray changes in the lungs, to be enumerated later. The youngest of the 22 children was but 8 months old. Only one child (aged 3¼ years) was more than 2½ years of age. The average age was 16 months. Thirteen were boys, nine girls. It so happened that all were colored. Although rachitis reaches grades of development in the black race in America rarely encountered in the white race, nevertheless, the condition described occurs as well in white as in black children. We have seen well-marked examples in white children in both this country and France. It is important to note that all 22 children were greatly under weight. One weighed 64 per cent under the normal standard for the age. Their average weight was 46 per cent under the average weights for healthy children of corresponding ages. All 22 children also were undersized. The body length averaged 88 per cent of the average for the corresponding ages. It is interesting to observe that the circumference of the chest was proportionately less than the length of the body. It was 83 per cent of the average for healthy children of corresponding ages. The circumference of the head averaged 96 per cent of the average of the standard measurements for the corresponding ages.

All 22 children also showed clinical evidence of rickets in parts of the skeleton other than the thorax. The deformities of the head and extremities were not particularly striking in all instances, however, and not in proportion to the deformities of the thorax. The majority had multiple fractures of the extremities, in a number of them not recognized until examined with the X-ray. A rhinitis with mucous discharge and resulting stenosis of the nasal passages was present in the majority of the children. In some, undoubtedly, it played a part in the causation of the inspiratory collapse of the thorax and hence in the development of the respiratory condition.

When viewed from in front, the deformed thorax appears smaller than it actually is, in part because of its peculiar shape, presently to be described, in part because of its situation between the disproportionately large rachitic head and distended abdomen. Roughly the deformed thorax may be likened to a blunt wedge, the sternum and adjacent portions of the costal cartilages corresponding to the narrow end of the wedge. The peculiarity in its shape is due to the fact that the antero-lateral portions have sunk inwards with the production of broad hollows, the deepest parts of which correspond to the sites of the costochondral junctions (Figs. 1 and 2). At the end of expiration, these depressions extend in a general way from the midaxillary line behind to within 2–3 cm. of the sternum, sometimes including, sometimes failing to include the nipple, and from the third or fourth rib above to the ninth below, where the thorax usually begins to flare outward over the abdomen. The upper part of the depression, which is narrow, is usually covered by the pectoralis major muscle. The deepest part lies at the sixth or seventh costochondral junction. The depression on the right side is in most cases larger and deeper than the one on the left, probably because the heart furnishes support to the thoracic wall on the left side. In the less severe forms there may be no depression on the left side but only flattening of the normal arch. The posterior angles of those ribs entering into the formation of the depression are much more bent than normal. Together they form a rounded ridge-like elevation 3–4 cm. broad, which projects laterally and sharply separates the back from the antero-lateral surface of the chest. Anteriorly this ridge slopes downward into the floor of the depression and forms its posterior boundary.

If the child can be made to sit up, it is at once apparent that the clavicles are exceedingly prominent. They are much bowed and stand out unusually far from the base of the neck. In a considerable number of cases they are the seat of fracture. The manubrium is prominent and appears to be elevated, as indeed does the whole thorax. In consequence of the high position of the thorax, the neck in front seems unusually short (Fig. 3). The sternum is thrust forward and is not infrequently curved or bent. Its inclination downwards and forwards is often much exaggerated. The prominence of the sternum, which might be compared to the inverted hull of a boat, is due to the fact that the ends of the ribs on the two sides of the chest are closer together than normal as the result of the collapse of the thoracic wall at the sides. A great increase in the arch of the costal cartilages is thereby produced. Occasionally on one or on both sides the costal cartilages are so greatly arched that those parts of them immediately adjacent to the sternum bow forward in front of the sternum and convert the latter into the floor of a groove. The manubrio-gladiolar junction often forms an obtuse angle pointing downward. In several of our cases the gladiolus was so inclined forward that it formed an angle of about 10° with the vertical, and in one instance an angle estimated at 25°. The xiphoid often points slightly backwards. The costal margins composed of the conjoined cartilages of the lower ribs and the ribs themselves usually flare outwards. On account of the hollows at the sides and the flaring of the lower margins the thorax when removed from the body bears a resemblance to a corset.

As already indicated, the thorax is not symmetrically deformed; indeed it is often markedly asymmetrical. The lateral depressions are never the same on the two sides. The broader the depression, the narrower the anterior surface of the thorax on that side and the sharper and more prominent the ridge made by the posterior arches of the ribs behind; the deeper the depression, the greater the flare of the costal arch below. The sternum itself often deviates from the median line and slopes toward one side or the other. The costal arch is always higher on one side than on the other and the flaring on the two sides unequal. If the child has sat, the vertebral column always exhibits lordosis in the interscapular region with kyphosis below and often well marked scoliosis. The back is rarely flat. If one of the lateral depressions is particularly wide and deep, the ribs involved pass backward and outward from the vertebral column instead of outward, causing the back on that side to project posteriorly. Moreover, the deeper the lateral depression in front, the narrower the back on that side behind (Fig. 4).

When fractures of the ribs are present, the distortion of the thorax may be greatly increased and the resulting deformities much more difficult to analyze. When the fractures are situated a short distance behind the costochondral junctions, small pits 1–1.5 cm. in depth are often formed. When the fractures are near the posterior angles of the ribs, these angles lose their rounded character. Since one rib derives support from another, often several ribs become fractured in series and, inasmuch as the points of fracture usually occur in line with each other, the resulting deformities appear as sharp ridges running obliquely down the side of the chest with a depression in front of them. It is of course impossible to do more than indicate the bizzare forms which the thorax may assume under the influence of the rachitic process with its resultant fractures.

When inspiration takes place in the deformed thorax just described, almost every deformity which has been mentioned becomes intensified. The depressions at the sides lengthen, broaden and deepen. The floor is carried backward and inward toward the vertebral column, in severe cases from 1–1.5 cm. and even 2 cm. As the lateral depression en-

larges, the posterior angles of the ribs involved narrow, and the whole posterior costal arch undergoes a displacement backwards. The prominence formed by the sternum and costal cartilages narrows as if squeezed together at its base. The manubrium moves slightly forwards and upwards. The lower end of the gladiolus remains stationary or is drawn backward; in some cases it moves slightly forwards as in older children with chicken-breast. Often the sternum describes see-saw movements with respiration, or with each inspiration the middle part of the sternum bows forward beyond its upper and lower ends. In general, it may be said that every hollow, every eminence, every peculiar curvature or inequality present during expiration becomes increased during inspiration.

The moment that the inspiratory effort is complete, the whole thorax snaps back into the expiratory position as if released from a spring.

If measurements of the circumference of the thorax are taken, it will be found that the circumference at the level of the nipple either increases to the extent of only 1 or 2 mm. during inspiration or is not increased at all or undergoes an actual loss (Fig. 5). In reality, however, it is impossible to obtain correct measurements of the variations in the circumference of the thorax during inspiration, because the tape bridges over the hollows of the sides. It is difficult to detect, anywhere in the diseased thorax, movements of the ribs occurring during inspiration which would bring about an actual increase in intrathoracic volume save the feeble upward and forward movements of the upper part of the thorax indicated by the slight movements in those directions of the manubrium. The evidence of the loss of intrathoracic space during inspiration in the collapse of the thoracic frame at the sides is so apparent as to leave little doubt that, the movement of the ribs alone considered, the net result of any given inspiration must be actual loss. Since the child remains alive, however, there must be some mechanism available through which the capacity of the thorax is increased with each inspiration. That mechanism must lie in the diaphragm. We shall return presently to the discussion of the diaphragmatic function.

The deformities and unusual movements of the thorax just described are brought about because the bony thorax has lost its rigidity. This loss of rigidity is the direct result of the failure of calcium deposition and, very probably, also, of resorptive processes in the bones themselves set in operation by the disease. The ribs, normally composed largely of inorganic material, firm and unyielding, become composed chiefly of organic material and are soft and yielding. The shafts of the ribs of some of the children, who have died, offered about the same resistance to bending as if they had been composed of pasteboard. In these severe cases fractures are almost regularly present. In one case we counted twenty. These further greatly impair the rigidity. The most extreme weakening at any portion of the costochondral arch develops at the costochondral junctions.

Under normal conditions the cartilage and shaft of the rib meet as do two accurately matched beams. The opposed ends of cartilages and ribs are slightly uneven and are to a certain extent mortised into each other. The union between cartilage and shaft is made firmer by the columns of calcified intercellular substance which, forming in the terminal zone of cartilage, extend like so many nails into the substance of the shaft. The normal costochondral junction is so strong that, when an attempt is made to break it, it is the shaft of the rib which buckles under the strain. In rickets a zone composed of osteoid tissue (bone tissue devoid of its normal content of calcium), connective tissue, blood vessels and degenerated and undegenerated cartilage in the form of bands and islands, gradually forms between the cartilage and shaft. This heterogeneous mass of tissue, known as the transitional zone or metaphysis, has no rigidity and is capable of offering about the same degree of resistance to any deforming force as connective tissue. As a result of forces acting upon the thorax, the weak metaphysis begins to yield almost as soon as it begins to form. The costal cartilages, wholly unaffected by the disease, retain their normal elasticity and, when forced inward during inspiration, tend always to spring back into their normal positions. The diseased ribs, however, never having possessed an elasticity at all comparable to that of the cartilage, lose the greater part of what was formerly theirs. As the result of the continuously repeated strain, to which they are subjected by inspiration, they become bent further and further inwards until finally their extremities lie internal to the cartilage. In the most severe forms of rachitic involvement of the thorax the costochondral junctions become so deformed that the end of the cartilage is no longer joined to the end of the shaft but to the outer side of the end of the shaft (Fig. 6). When the costochondral junctions have become affected to this extent, all rigidity has long since disappeared; indeed the shaft and cartilage have become so loosely attached to each other that a considerable degree of motion can readily take place between them. In this state the costochondral junction really constitutes a false joint.

The forces that operate to produce deformity in the thorax are the negative pressure in the thoracic cavity existing during inspiration, the elasticity of the lungs, the pull of the diaphragm and of the various voluntary muscles passing to the thorax from the abdomen and neck. To this list of forces might be added two of less importance, gravity and intra-abdominal pressure. With the exception of gravity, the forces mentioned are applied either solely or in by far their greater part during inspiration. The normally curved, strong, resilient thorax of the healthy child withstands them completely. When the negative pressure within the normal thorax reaches extreme heights during inspiration as in laryngeal stenosis, it is always the costal cartilages of the lower ribs and the lower part of the sternum which yield and are sucked backwards. The ribs and costochondral junctions unaffected by disease are capable of withstanding great strain. The diseased ribs and costochondral junctions which form the wall of the rachitic thorax cannot do so. The forces brought to bear upon them gradually cause the shafts of the ribs to sink inwards anteriorly, carrying the cartilages with them. The

muscles of the neck attached to the first and second ribs and clavicles pull these bones upwards. The abdominal muscles draw the lowermost ribs downwards. Intra-abdominal pressure, if in excess of the normal, forces the lower aperture of the thorax outward. The weakened thorax has become the prey of every force exerted upon it.

Reference has already been made to the fact that the circumference of the thorax is diminished, remains stationary, or is increased very slightly during inspiration. Although it is impossible to determine the point, it is highly probable that as the result of thoracic respiration alone an actual loss of intrathoracic space would occur during inspiration. It is the action of the diaphragm that makes life possible. But the diaphragm can act to advantage only when its points of attachment remain firm. In the weakened thorax of rickets the diaphragm expends part of its energy in drawing its own points of attachment inward. In so doing it diminishes the extent of its descent and impairs by just so much its power to increase intrathoracic space. This is not all. Owing to the deformity of the thorax, the diaphragm must tend to remain even in expiration in a state of partial inspiratory contraction. When inspiration takes place, it must commence its contraction from a position of partial contraction and is able, therefore, to accomplish only the last part of the work which, as a muscle in a state of normal relaxation at the outset, it would be capable of accomplishing. It is quite possible also that the contractile power of the diaphragm is impaired as a result of the rickets itself.

The changes just described in the thoracic wall must inevitably lead to changes in the thoracic contents. When the thorax is opened, attention is immediately attracted to two striking conditions (Fig. 7), the knuckle-like enlargements at the costochondral junctions which project a considerable distance into the thoracic cavity, and the grooves, one on the external surface of each lung not far from the anterior border, running in a direction from above downward, outwards and backwards. If the lungs are pulled forward into their anatomical positions, the grooves on the surface of the lungs come to lie directly opposite the lines of the deformed costochondral junctions, and the relationship between the two is clearly shown by the presence of depressions in the grooves exactly corresponding to the costochondral prominences themselves. In reality the depressions are moulds of the prominences. When the lungs are removed from the thorax, it is seen that they are far too small for the size of the child. The right lung is usually proportionately smaller than the left. It can then be seen that the grooves divide the external surface of each lung in such a way that roughly one quarter lies in front and three-quarters behind. That part of the lung in front of the groove is for the most part emphysematous, while that part lying behind it is generally dark bluish in color and of a firm consistency. Obviously it is atelectatic. It is not entirely atelectatic, however, for interspersed with the collapsed pulmonary tissue is tissue in a state of emphysema. In some cases the greater part of one lobe is atelectatic. On section through the lung, it can be seen that the floor of the groove is composed of atelectatic tissue and that the thickness of the lung at the groove has been reduced to 2–3 mm. Further, the part lying in front of the groove, though chiefly of emphysematous tissue, contains scattered areas of atelectasis, while behind the grooves atelectasis so predominates over emphysema that the greater part of the lower lobe appears to be collapsed (Fig. 8). Areas of lobular pneumonia are almost always present. They are not large and frequently cannot be recognized on gross examination. The cut surface almost invariably exudes serum, showing the presence of edema. No pleurisy is present. In some cases, however, adhesions are found between the visceral pleura and the sharp points at the summits of the knuckle-like enlargements of the costochondral junctions (Fig. 6). These points are formed by the projection of the pointed shafts of the ribs through the rachitic metaphyses. The adhesions are produced by the irritation of the sharp bony points against the pleural surface. On microscopic examination of the lungs atelectasis is the predominating and essential condition in the more solid parts. Evidences of inflammation can usually be found, but large areas of the lungs are not the seat of pneumonic consolidation.

As a result of the deformity, the thorax becomes divided into two communicating chambers (Figs. 9 and 10), an anterior one bounded in front by the sternum and abnormally arched costal cartilages and at the sides by the projecting walls of deformed costochondral junctions, and a posterior one bounded by the rest of the thorax. In the anterior chamber the heart is imprisoned. The apex of the heart always lies against the barrier of the costochondral junctions on the left, usually at the level of the fourth or fifth rib. The distended right auricle, forming the right border of the heart, reaches to within a short distance of the costochondral junctions on the right side.

On removal of the heart and exposure of its cavities, that of the left ventricle appears to be normal in size and to have normally developed internal musculature, but that of the right ventricle often appears dilated and hypertrophied (Fig. 11). The latter stands open and is half again as large as the cavity of the left ventricle. The columnæ carneæ are thick and flattened out against the ventricular well. Though the weight of the body is considerably less than the average for the age, the weight of the heart may equal or even exceed the average for the age. The wall of the left ventricle is of normal thickness; the wall of the right ventricle is frequently much thickened. Hypertrophy of the heart is, therefore, present, and the part giving evidence of it is the right ventricle. In one of our cases there was hypertrophy of the left ventricle as well. Upon the surface of the liver can invariably be seen the imprints of the 7th, 8th and 9th costal cartilages impressed through the diaphragm. The intestines are usually distended.

A number of months of progressive rickets must elapse for the costochondral junctions and shafts to become so weakened that the characteristic clinical condition develops. The clinical picture in well marked cases is as follows: The child lies on the back with arms at the sides and legs semiflexed and rotated outward (Fig. 1). The external rotation of the legs,

due to the laxity of the muscles and ligaments and probably also to curvatures in the bones themselves, is so extreme that, though flexed, the knees lie in contact with the bed. The child lies remarkably still, never attempting to change the position of the body and only occasionally turning the head or shifting slightly the extremities. The head shows the marks of rickets, as do the extremities, and the forehead is frequently covered with sweat. The large head appears as if placed directly on the thorax. The chin rests against the upper part of the chest and a deep fold in the soft parts alone indicates the neck. The expression is anxious. The mucous membranes may be slightly cyanotic. The dyspnea is extreme; the respirations are from 60 to 100 a minute—and in one case 150. They are accompanied by dilatation of the nostrils and an expiratory grunt. A cough occurs from time to time, usually singly, separated from succeeding coughs by one or more respirations. Sometimes, however, the cough comes in short paroxysms which seem to distress and terrify. The respirations are shallow. With each inspiration the thorax contracts. When the child coughs its collapse is doubled. As the thorax sinks in with each inspiration, the abdomen expands. Indeed the appearance suggests that the contents of the thorax are being rhythmically squeezed into the abdomen rather than that the lungs are being inflated and the normal process of respiration is taking place.

On account of the almost continuous movement of the thorax, especially marked and jerky in the region of the costochondral junctions, the determination of the location of the apical impulse of the heart is exceedingly difficult. It may be impossible to locate it. Usually it can be seen in the fourth or fifth spaces or in both about 1 cm. beyond the nipple line, often in or just beyond one of the depressions at the costochondral junctions. In some cases the apex impulse is strong and visible over rather a wide area and can be felt[1] as well as seen. There may be marked epigastric pulsation, or pulsation over the entire left side of the precordium. It is impossible to determine the location of the left border of the heart by means of percussion on account of the proximity of the enlarged costochondral junctions and impossible to determine the right border by that means with any degree of accuracy, not only on account of the changes in curvature and the abnormal character and asymmetry of the thoracic wall already described, but on account of the abnormal conditions in the lungs themselves. If, however, the costochondral junctions on the two sides lie close together, it may be assumed that the right border of the heart is close to the wall formed by the costochondral junctions of the right side, that is, the heart occupies the entire space between them. The action of the heart is very rapid. Unless cardiac disease unrelated to the rickets is present, the sounds are clear. We have never observed an accentuation of the pulmonic second sound which could be regarded as pathological.

In the examination of the lungs it is difficult to obtain accurate information concerning pathological conditions by means of percussion on account of the abnormal physical conditions in the thorax itself. Changes in percussion resonance are always present and easily demonstrated, but are not necessarily produced by changes in the lungs. Usually an impairment of the percussion note can be found in some portion of the chest, but in our experience it is never great. In not one of our cases was there well marked dulness. The breath sounds also are subject to great variation in different parts of the chest. In the lateral depressions they are often of an exaggerated vesicular character. Invariably in some parts of the thorax the breath sounds are greatly diminished and may be scarcely audible. The respiratory murmur may be higher pitched than normal, expiration may seem prolonged but loud bronchial breathing is never present. Râles can always be heard. They may be abundant or few, fine or moist, but are never evenly distributed throughout the lungs. They are most apt to be numerous over the bases of the lungs. The liver edge can be felt, usually 4 cm. or more below the costal margin. The spleen is often palpable.

The X-ray findings in the chest are characteristic (Figs. 12 and 13). The ribs look extraordinarily slender and have a porous appearance. They are bent and have lost their normal inclination and parallel arrangement. Fractures, even though present, are not evident. In cases of long standing rickets, in which the thorax has finally acquired rigidity, the ribs may appear greatly thickened. If scoliosis of the vertebral column is present, it is of course visible. The heart seems disproportionately large for the size of the thorax, often appearing enormous in comparison with the latter. On both sides of the heart are longitudinal shadows with indefinite margins 1–1.5 cm. wide (in X-ray plates of ordinary magnification). They extend downwards, at the same time curving slightly outwards, from a point at about the level of the second or third thoracic vertebra to the shadow of the liver and diaphragm below. The one on the right side begins near the shadow of the vertebral column above and is separated by a slight interval from the right border of the heart below. The one on the left side merges as it descends with the shadow of the heart or skirts the left border of the latter. The two longitudinal shadows are produced by the atelectatic lung beneath the costochondral junctions. If the costochondral junctions themselves cast shadows, as they do when healing has commenced, these also share in the formation of the longitudinal shadows. Elsewhere the lungs have an irregularly hazy appearance, due to the existence of scattered areas of atelectasis and also in some cases to scattered areas of an inflammatory nature. The lungs external to the longitudinal shadows never appear clear and never show the large dense clearly outlined shadows of complete consolidation. The

[1] Wherever on the chest wall the apex impulse of the heart may appear to be, however, there can never be any doubt in regard to the situation of the apex of the heart itself. The heart is always imprisoned in the space bounded in front by the sternum and cartilages and at the sides by the wall of costochondral enlargements which project into the cavity of the thorax. Beyond the barrier of the costochondral junctions the apex of the heart never passes. Any impulse which the heart can communicate to the chest wall by means of its apex is caused by the impingement of the apex against one of the costochondral junctions in the barrier.

X-ray findings in the thorax in these cases, though quite typical, are often erroneously considered to indicate tuberculosis.

The clinical picture just described greatly resembles that of pneumonia and is usually considered such. Yet the temperature is rarely greatly elevated, and is not infrequently normal (Fig. 14). In one of our cases the temperature was normal for the first seven days in the hospital. It began to rise only two days before death, reaching at that time 101° F. During the nine days of observation the respirations were never less than 60 and averaged between 80 and 100 to the minute. The leucocyte count also is low. In the 22 cases the leucocyte count varied between 4000 and 18,000 and averaged 11,000. In some of the severest cases the leucocyte count did not exceed 8000. Another fact of importance is that the symptoms remain but slightly modified after the evidences of infection have disappeared. The clinical picture just presented is caused by the loss of the rigidity of the respiratory frame, the result of the rickets. The infection is subsidiary; it furnishes the extra burden which the enfeebled thorax cannot bear.[2]

The sequence in the development of the condition seems to be as follows: The rachitic thorax loses its rigidity, yielding during inspiration simultaneously at costochondral junctions and shafts. As the rigidity of the thorax becomes diminished, the depressions at the sides, which at first were present only during inspiration, persist during expiration. As the bones progressively lose their elasticity, the dynamic deformity gradually becomes static as well. Thoracic respiration is at first slightly, later profoundly, affected. Coincident with the impairment of thoracic respiration diaphragmatic respiration is enfeebled. The efficiency of each inspiration, therefore, is diminished. A compensation is attempted in an increased frequency of respiration and in an increased force. But the greater the force applied, the greater the collapse produced. Nature's efforts in large part defeat themselves. The diaphragm pulls its attachments yet further inward, and the accessory muscles draw the bones to which they are fastened out of position without moving or giving stability to the thorax as a whole. Owing to the progressive reduction in the size of the thorax and its loss of power to expand, the lung begins to become atelectatic. One of the parts early affected is that pressed upon by the costochondral junctions. As the pathological process progresses, the atelectasis increases; the pressure in the pulmonary circulation rises; and hypertrophy of the right heart follows. If the rigidity of the ribs becomes still further impaired, a point is reached at which the disabled respiratory machine is just sufficient to perform its work. If now a slight additional burden is put upon it in the form of an infection, it becomes insufficient. Nature resorts to the only expedient possible; the frequency of respiration becomes enormously increased; the clinical condition above described ensues.

The course in such cases as we have been discussing is subject to great variation. In one group of children the respiratory symptoms and distress became progressively worse until death intervened. In another group the condition remained essentially unchanged over a long period. From time to time the temperature would rise to 102° or 103° F. without apparent cause or any increase in the number of white blood cells. It would remain elevated for a day or two, and then fall to normal again. With the rise in temperature, the respiration would become more rapid and the respiratory distress more acute. Death often seemed to impend, but with the fall in temperature the condition of the child would again ameliorate. This state of affairs, characterized by alternating exacerbation and decline of symptoms, has continued for weeks. Such children after spending several weeks in the hospital improve sufficiently to leave, but in a short time are readmitted in the same dangerous condition as before.

If a child does not die, he may gradually improve, even though the rickets continues and lime salts are not deposited in the bones. The shafts of the ribs and the costochondral junctions ultimately may acquire sufficient strength to withstand the forces exerted upon them. Given time, osteoid tissue is produced in such quantity both in the cancellous tissue, under the periosteum and in the metaphysis itself as to supply the requisite rigidity. Even though the costochondral junctions remain weak, respiration will take place in a normal or approximately normal manner provided the shafts of the ribs acquire sufficient strength. Ultimately a rigidity may be furnished the ribs through an overproduction of inferior material. It is due to this fact that we do not often see cases, such as have been described, in children older than 2½ years. Extreme deformities of the thorax are common enough in children 3 and 4 years of age, but the deformed thoraces will almost always be found to be rigid, and the bones of such children, not only the ribs but also the bones of the extremities, will be seen to be enormously thickened when studied by means of the X-ray. The children of our series whose thoraces were the weakest were children from 8 months to 1½ years old. *For the comprehension of the condition it is necessary to realize that deformity and normal thoracic function are compatible, in other words, that the element of danger is not the deformity but the loss of rigidity of the thoracic wall.*

With the aid of cod-liver oil calcium salts can be deposited in the rachitic metaphyses and shafts and a true healing of the disease accomplished. In spite of this, however, the deformities will persist for months or years. But gradually, we cannot say how rapidly, the deformities of the thorax are outgrown. In adults and in older children malformations of the thorax, such as we have described, are never seen. Changes in the extremities such as bow-legs and knock-knees are common enough in adult life. It is rare, however, to find a rachitic adult showing more than slight evidence of previous thoracic deformity. The pigeon-breast of older children and adults is usually a late development for which continued obstruction in the upper respiratory tract rather than rickets is responsible.

In regard to the mortality we cannot say a great deal. Out of a series of 22 well marked cases studied in the hospital, we

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[2] See legend explaining Fig. 5

know that 11 have died.[3] It is probable that others of the 22 children discharged have died outside the institution as the result of the rachitic involvement of the thorax. The mortality in the severely affected cases is undoubtedly exceedingly high.

Rickets is often looked upon as a disease that in itself does not threaten life. It does threaten life, however, and may be the direct cause of death when it deprives the thorax of its rigidity. It increases the danger to life from all respiratory infections, *e. g.*, tuberculosis, pneumonia, at that moment when it causes the thorax to yield during inspiration.

Treatment must be prophylactic. Such extreme rachitic changes as we have described should never develop. To bring rickets to a sudden termination is in the present state of our knowledge out of the question. To offer mechanical support to the yielding ribs through orthopedic devices seems impracticable. At present the only course is to administer cod-liver oil in moderate doses, to avoid infection and those things which give rise to abdominal distention.

Before concluding this paper, we wish to point out that we have found in the rats rendered rachitic by means of faulty diets[4] a condition identical with that just described in children, the subjects of the most severe rachitis. In the course of severe rickets in the rat, growth becomes greatly inhibited. The weight falls far behind the weight of the healthy animal and finally remains stationary. Growth in length is also much retarded. Multiple fractures of the ribs develop. As a result of the weakening effects of the disease upon the bones, the shafts of the ribs and the costochondral junctions lose their rigidity. The thorax collapses at the sides and the costochondral junctions become deformed and displaced well into the interior of the thorax. The costal cartilages join the shaft often at right angles and are attached not to the ends but to the outer sides of the shafts near the end. The deformed costochondral junctions of the rachitic rat are the deformed costochondral junctions of the rachitic child in miniature. The sternum and adjacent portions of the cartilage are thrust forward and the sternum acquires an abnormal forward inclination as it descends, exactly as in the human being. As the result of the displacement of the costochondral junctions inward, the thoracic cavity becomes divided into the two chambers already described (Fig. 15). The heart is imprisoned in the anterior chamber, which it completely fills. The lungs are partially collapsed and show bands of atelectasis in those parts subjected to the pressure of the costochondral junctions, and scattered areas of atelectasis elsewhere.

With each inspiration the whole lateral wall of the thorax sinks forward. Respirations are enormously increased. In one rat, the subject of advanced rachitis of the thorax, the respirations numbered 220 to the minute. If the faulty diet is continued a sufficiently long time, the animals die. Whether they die, however, as the result of the loss of rigidity of the thorax or from other causes, we have not tried to determine.

[3] One of the 22 cases is a child just admitted to the hospital. The outcome is uncertain.

[4] Jour. Biol. Chem., 1921, XLV, 333.

DESCRIPTION OF PLATES

Fig. 1.—H. B., aged 17 months. The depression at the side of the thorax is well marked, as is also the rounded ridge created by the posterior angles of those ribs forming the floor of the depression. The rounded elevation formed by the posterior angles of the ribs is the posterior boundary of the depression, and sharply separates the back from the antero-lateral surface of the deformed thorax. The forward inclination of the sternum and the recession of the ensiform cartilage are well shown. The picture of the child is quite typical. Though the legs are flexed, the knees are in contact with the surface of the table.

Fig. 2.—Photograph of E. D., aged 3¼ years. The collapse of the sides of the thorax is extreme. The left side is more sunken than the right as the result of fractures of the ribs.

Fig. 3.—Photograph of S W., aged 3½ years. The deformity of the thorax is typical. The photograph is reproduced to show the high position of the thorax. The manubrium immediately underlies the chin. The neck is marked by a fold. The deformity of the vertebral column is characteristic of rickets. In reality, the child no longer belongs to our series of cases, because the ribs and costochondral junctions had acquired sufficient rigidity through an overproduction of osteoid completely to withstand the forces exerted upon them. The bones of the extremities had become enormously thickened.

Fig. 4.—R. W., aged 28 months. The depressions at the sides of the thorax, when marked, narrow the back. At the level of the scapulæ there is lordosis, but below in the region of the lower dorsal and upper lumbar vertebræ a well marked rachitic kyphosis. The abdomen is large. The child is obliged to support the body with the hands in order to sit. In the most severe cases the child is too weak to sit.

Fig. 5.—Tracings from chest and abdomen of J. R., aged 16 months, to show that the circumference of the thorax may actually undergo a decrease during inspiration. Thin pneumatic bags were placed, one around the middle of the chest so as to cross the lateral depressions, the other around the abdomen. Both bags were covered with thin bands of canvas, filled with air and connected with tambours.

Tracing 1 records the movements of the thorax. Inspiration begins at a and ends at b. The tracing shows that a progressive diminution in the size of the thorax, measured in the horizontal plane, occurs during inspiration. The sharp rise in the tracing marked x was produced as the result of the sudden recoil of the thorax occurring immediately after the inspiratory effort was finished. The thorax seems to snap from the inspiratory to the expiratory position at the end of inspiration like a suddenly released spring.

Tracing 2 shows the increase in the circumference of the abdomen during inspiration. a marks the beginning of inspiration, b its termination.

The loss of rigidity of the thorax was so great that the slight pressure from the inflation of the pneumatic bags was enough to cause asphyxia and cessation of breathing. The child was resuscitated with great difficulty and the attempt to obtain further tracings abandoned.

It is appreciated that the records obtained by the method described do not give exact measurements of the variations in the size of the thorax during respiration. They show definitely, however, that in severe rickets the thorax may undergo a marked decrease in size at the level of the lateral depressions while inspiration is taking place.

The tracings were obtained through the kindness of Dr. Harold L. Higgins.

Fig. 6.—A section taken in the horizontal plane through the enlargement formed by the junction of the cartilages and rib from the thorax of A. H., aged 14 months. The section was stained with hematoxylin and eosin.

a—shaft.

b—undifferentiated cartilage.

c—cartilage of the proliferative zone.

d—the transitional zone or metaphysis lying between the proliferative zone of cartilage and the shaft. In this zone tongues and bands of cartilage (like the rest of the cartilage appearing black) are interspersed with composites of tissue made up of connective tissue, blood vessels ending in large tufts, degenerated cartilage in the various stages of metaplasia into osteoid, and osteoid itself.

e—connective tissue and osteoid filling in the crevice between the cartilage and transitional zone on the one hand and the outer surface of the shaft on the other.

f—a piece of the visceral pleura which adhered to the pointed end of the shaft protruding through the costochondral enlargements. The visceral pleura rubs against the sharp point of the shaft and finally becomes attached. Although the section shows the tag of visceral pleura, it misses the protruding end of the shaft which lay in another plane than the plane of the section. The cartilage and shaft meet at right angles. The cartilage is joined to the shaft on its outer side.

Fig. 7.—The thorax of W. M., aged 16 months, opened at autopsy. The costal cartilages have been cut through near the costochondral junctions and removed together with the sternum.

a—enlarged costochondral junctions; the cut ends of the cartilages are visible. The cartilages meet the shafts in some instances at right angles. The bulbous character of the enlargements of the costochondral junctions and the knuckle-like projections which they make into the interior of the thorax are well shown. The sharp ends of the shafts of the ribs can be seen protruding through the inner side of the enlargements and forming their summits.

b—grooves on the external surfaces of the lung produced by the pressure of the enlarged costochondral junctions. The thoracic wall has been retracted so that the grooves are exposed to view.

c—emphysematous lung in front of the grooves. As a result of the partial compression of the bronchi by the enlarged costochondral junctions the discharge of air from those portions of the lung internal to the grooves is interfered with. A condition of emphysema is therefore produced.

d—a large part of the left lung is atelectatic.

Fig. 8.—A section taken through the lung of A. H., aged 14 months, to show the combination of atelectasis and emphysema.

a—atelectatic lung underlying the enlarged costochondral junctions.

b—atelectatic lung bordering on the vertebral column.

c—pulmonary tissue in a state of emphysema.

"The left lung is apparently diminished in volume. A vertical groove traverses the lateral aspect of the upper lobe in such a way as to separate the anterior quarter from the posterior three-quarters. The groove is formed by atelectatic tissue. In front of the groove the lung is downy and yellow, in a state of emphysema. Behind the groove the lung is pink, showing areas of emphysema and some atelectasis. The groove traverses also the lingula, separating the tip which forms a rounded inflated mass by a neck of atelectatic tissue from the portion behind. The paravertebral parts of both lower and upper lobes are in a state of atelectasis. The surface is depressed and dark blue and the interlobular tissue is visible. Scattered elsewhere through the lower lobe are bluish depressed areas of atelectasis."

"The right lung is also reduced in bulk. The groove marked by the atelectasis is even more conspicuous. Where it crosses the middle lobe it is 2 cm. in width and the floor thereof 2 mm. in thickness. That part of the middle lobe which lies in front of it is distended to its utmost capacity and is downy and white. The grooving of the upper lobe is very pronounced. Fully one-half of this lung appears collapsed. All that part in the paravertebral groove and by far the greater part of the rest of the lower lobe is atelectatic. The apex and that part of the lower lobe which lies in front of the groove above mentioned are extremely distended. Viewing the lung from the inner side and below, it is seen that the atelectasis occupies large areas, tending to be most marked in the region of the hilus—but extending to the periphery." [Quotation from autopsy protocol.]

Fig. 9.—Views of the normal thorax; *a*—cut across at the level of the sixth rib and viewed from below; *b*—viewed from in front.

Fig. 10.—Views of the deformed thorax in rickets.

a—a not very severely deformed thorax cut across through the sixth rib and cartilage, viewed from below. Note the asymmetry. The projections inward of the deformed costochondral junctions divide the thorax into two chambers. In the anterior one the heart is contained. As usual the recession inward of the thoracic wall is greater on the right side than on the left, and the arch of the ribs and cartilages correspondingly increased. Attention should be called to the fact that the thorax is in the position in which the deformities are least marked. If the drawing had been made from the thorax in the position of inspiration instead of expiration, the degree of deformity would have been increased almost two-fold. The costochondral junctions would have been 1 cm. or more nearer the vertebral column; the anterior chamber would have been correspondingly narrowed and the arching of the ribs increased.

b—The thorax of the same child, viewed from in front. The malformation is more marked on the right side than on the left. The enlargement of the costochondral junctions is very slight externally but well marked on the inner surface of the thorax. Owing to the collapse of the thoracic wall inwards and backwards toward the vertebral column, the costal margins have been drawn upwards and backwards over the surface of the liver, so that a much larger part of the latter is uncovered than under normal conditions.

c—the junction of cartilage and shaft of the rib viewed from in front. The cartilage has been twisted about probably as the result of the rotatory movements to which the shaft of the rib is subject, so that it no longer lies in line with the shaft even in the horizontal plane. The enlargement is much greater on the inner than on the outer side.

d—junction of cartilage and shaft seen from below. The projection of the sharp end of the shaft through the enlargement formed by the abnormal junction of cartilage and shaft is well shown. The projecting point of the rib forms the summit of the enlargement.

Fig. 11.—Photograph of the heart of A. H., aged 14 months.

a—left ventricle;

b—right ventricle. Note the hypertrophy of the internal musculature of the right ventricle.

"Right chamber shows great hypertrophy and is dilated. Its wall measures 3 to 5 mm. in thickness. The wall of the left ventricle close to the aortic ring measures 1 cm. The internal musculature of the left ventricle seems normal. The internal musculature of the right ventricle is hypertrophied. Valves normal. Weight of heart 85 gm." [Quotation from autopsy protocol.] (Body weight not recorded.)

Fig. 12.—X-ray photograph of chest of C. H., aged 10 months. The ribs appear extremely slender and porous. They do not show the normal inclination and parallel arrangement. Some of them are greatly bent. On either side of the shadow cast by the heart are the longitudinally directed shadows cast by the strips of atelectatic lung underneath the enlarged costochondral junctions. The longitudinal shadow on the right side is separated from the shadow of the right border of the heart by a just perceptible interval. The shadow on the left side skirts the left border of the heart above and merges with it below. Evidently the heart extends from one row of costochondral junctions to the other. The lungs beyond the longitudinal shadows have a hazy appearance. The evidences of rickets in the humeri and scapulæ are well marked. The X-ray photograph is typical.

Fig. 13.—The X-ray photograph of thorax of V. F., aged 17 months. The heart shadow is well defined and large in comparison to the size of the thorax. On the right side of the heart shadow and on the left side are shadows caused by the deformed chest wall. The sides of the chest have sunken to such an extent that the portions of the thoracic wall forming the anterior parts of the depressions lie in the antero-posterior plane, that is, parallel to the Roentgen rays in-

Fig. 1.

Fig. 2.

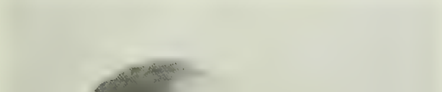


Fig. 3.

Fig. 4.

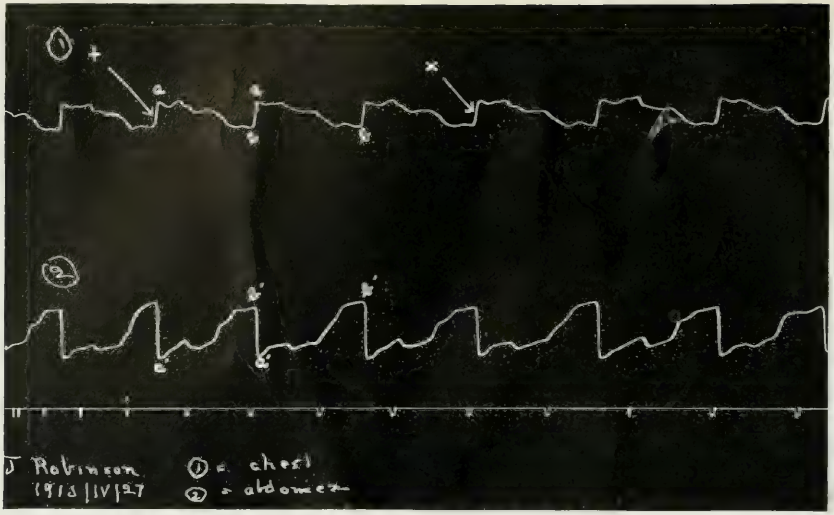


FIG. 5.

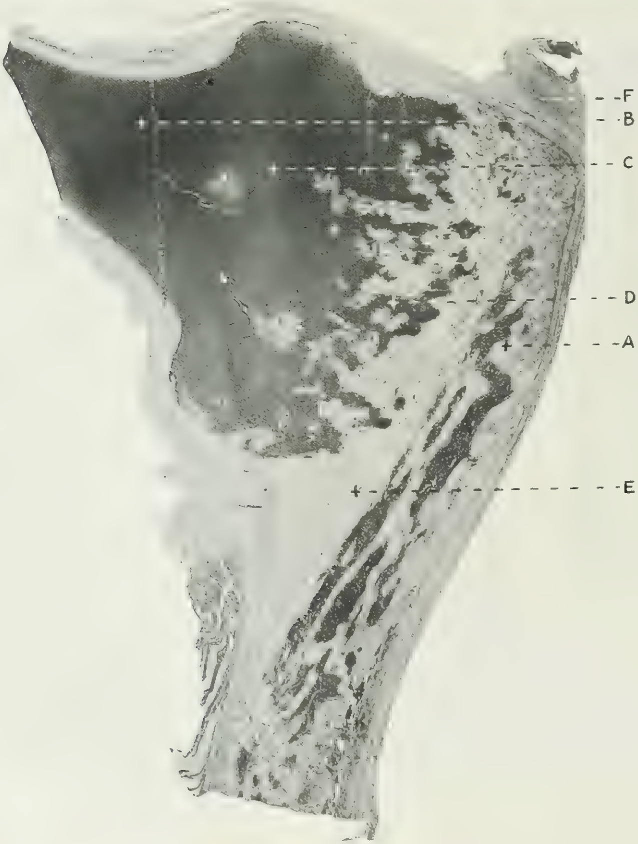


FIG. 6.

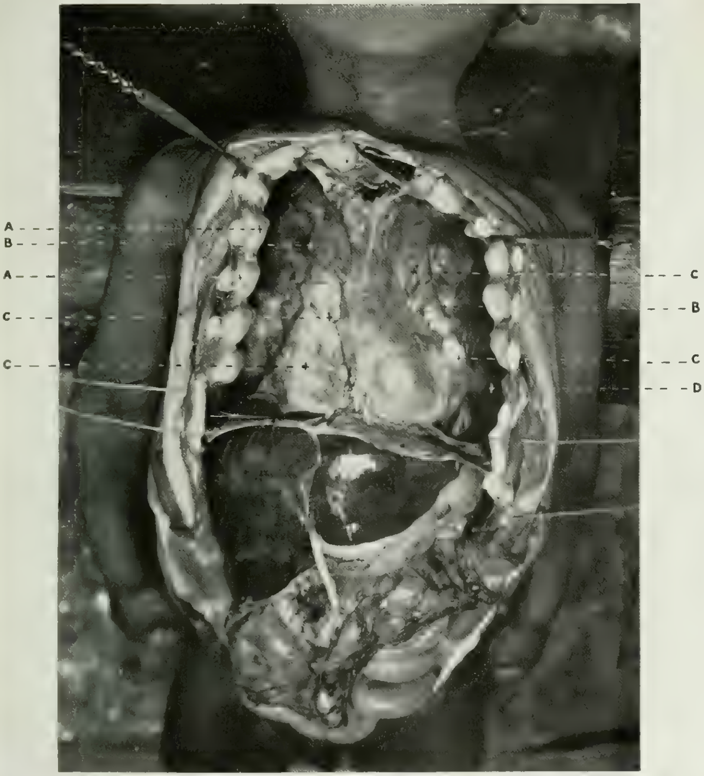


FIG. 7.

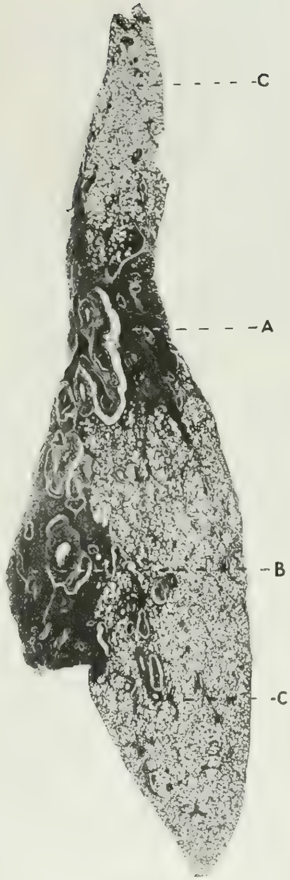


FIG. 8.

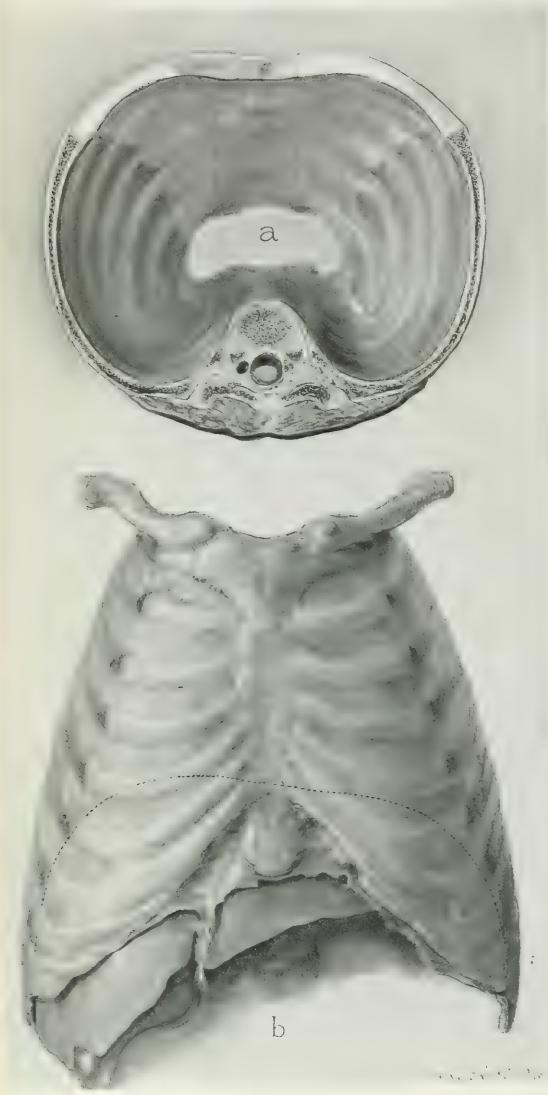


Fig. 9.

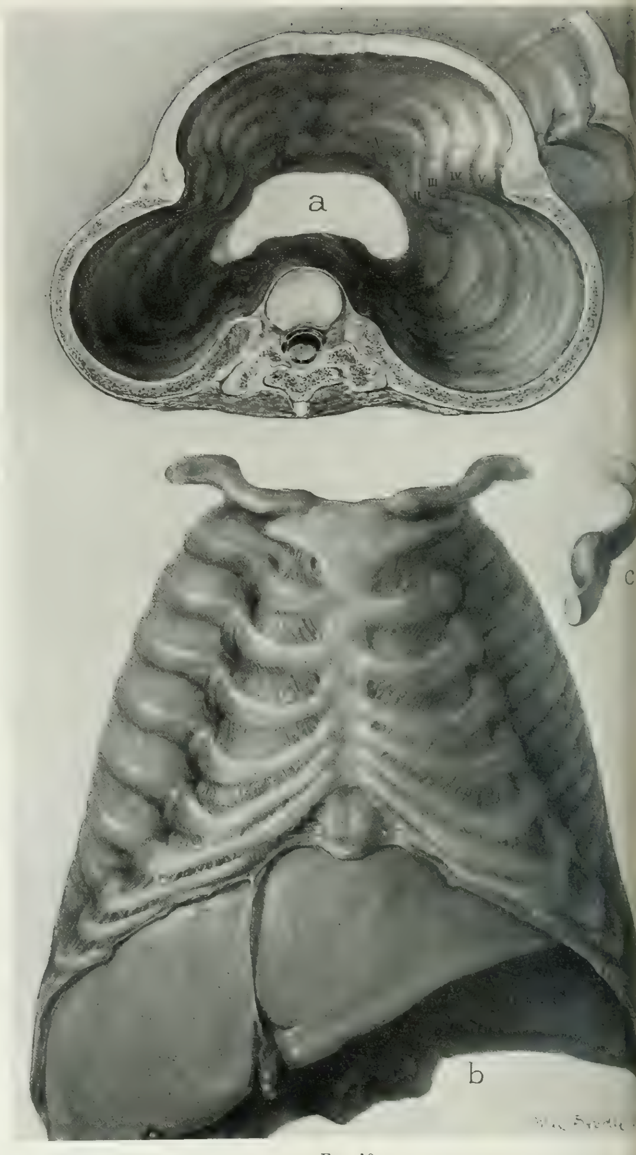


Fig. 10.

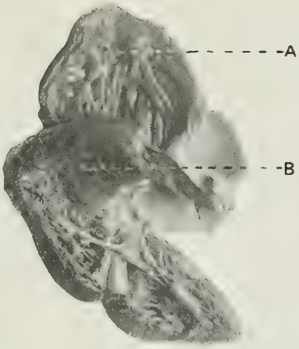


FIG. 11.

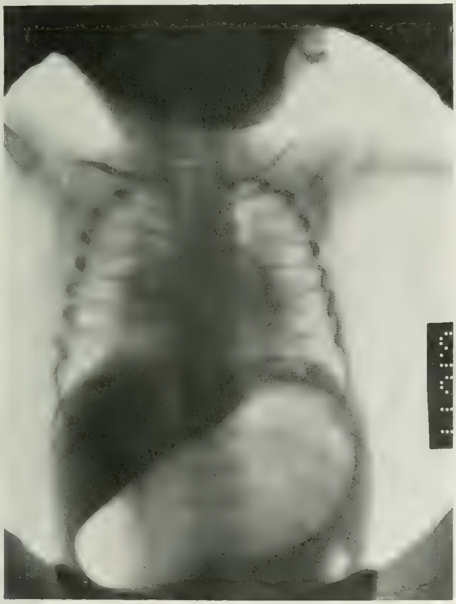
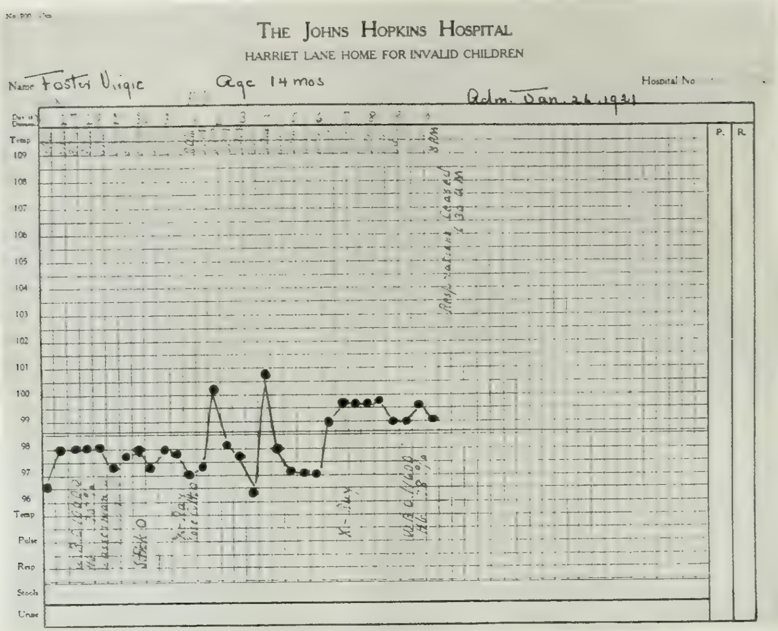


FIG. 12.

FIG. 13.


THE JOHNS HOPKINS HOSPITAL
HARRIET LANE HOME FOR INVALID CHILDREN
Name
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Hospital No
Adm. Jan. 26. 1921
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Temp
Pulse
Resp
Stools
Urine
P.
R.

FIG. 14.

FIG. 15.

stead of perpendicular to them. The shadow on the right side completely outlines the anterior margin of the depression. The shadow on the right side cast by the atelectatic lung underneath the enlarged costochondral junctions is merged with the shadow cast by the deformed chest wall while on the left side the shadow is continuous with the shadow cast by the latter. The heart must touch the row of costochondral junctions on the left side and extend almost to the row on the right. The ribs have a porous appearance and are abnormally bent. Scoliosis of the vertebral column is present.

The X-ray photograph in this case, showing so plainly the deformity of the chest wall, is unusual. It is reproduced for that reason.

Fig. 14.—Temperature chart of V. F., aged 17 months. Throughout the period of observation in the hospital the respirations during waking or sleep were never less than 60 to the minute. The thorax was greatly deformed and weakened. With each inspiration the collapse of the sides was extreme. The physical signs in the lungs were characteristic. There was no evidence of a pneumonic process either on physical examination or on examination with the X-ray. The respirations became more and more labored. Death seemed to occur from exhaustion.

Fig. 15.—A segment of the thorax of a rachitic rat viewed from below. Note that the costochondral junctions form marked prominences internally and divide the thorax into two chambers. In the anterior chamber the heart is, so to speak, imprisoned, exactly as in the case of the human subject. Note the abnormal curvature of the shafts of the ribs, and the asymmetry.

DIPHTHERIA BACILLUS CARRIERS

A REPORT OF CONDITIONS FOUND IN AN ORPHAN ASYLUM

By W. L. Moss, C. G. Guthrie and J. Gelien

(From the Division of Clinical Pathology of the Medical Clinic, The Johns Hopkins University and Hospital)

During an investigation of diphtheria bacillus carriers in the winter and spring of 1912 * throat cultures were taken from 800 children attending one of the public schools of Baltimore. From the carriers disclosed by the examination of these cultures, 50 were selected at random for further study. Throat cultures were taken from this group of 50 carriers at intervals of two weeks for ten weeks and a fortnight later cultures were taken from the entire 800 children. There were thus seven observations on 50 of the children and 2 on the remaining 750 over a period of three months. At the time of each of these seven observations, the condition of the throat was noted and the temperature was taken at each examination except the first. An attempt was made to isolate diphtheria bacilli from each of the positive cultures encountered, and the strains isolated were then tested tinctorially, culturally and for virulence. Some weeks after the last series of cultures, a systematic effort was made to determine the relation of the carriers found to the community at large. The detailed results of this investigation may be found on reference to the paper cited above but are summarized briefly here.

1. The first examination in February, 1912, revealed 85 carriers among the 800 children, or 10.62 per cent. Re-examination of the same 800 children three months later showed 69 carriers, or 8.62 per cent. It was a striking fact, however, that only 10 of the children gave positive cultures at both examinations. The number of children yielding positive cultures at either one or the other examination was 144, or 18 per cent.

2. Definite statements could not be made with regard to the actual duration of the carrier state in the 50 carriers selected for repeated study, but our findings indicated a progressive diminution in the number of positive cultures found at the successive examinations and at the last examination only six showed diphtheria organisms.

3. The diphtheria bacilli found in the throats of these carriers were typical Klebs-Loeffler organisms, but many of the strains were non-virulent. The non-virulent strains apparently differed from the virulent ones only in their ability to produce toxin. Since the other members of the diphtheria group encountered in the throat were readily distinguished from *B. diphtheriæ,* they caused very little difficulty in diagnosis and were considered of no pathogenic importance.

4. Virulence tests showed that only about 11 or 12 per cent of the carriers which we studied harbored virulent organisms and that of the total positive cultures obtained only about 10 or 11 per cent were virulent.

5. No convincing proof of a change in virulence of the organisms in the throat was obtained.

6. To quote directly concerning the relation existing between the presence of diphtheria bacilli and pathological throat conditions other than clinical diphtheria: "Our general impression upon this point is that the mere presence of diphtheria bacilli in the throats of carriers is not responsible for any objective pathological condition whatever, and does not cause an elevation of temperature. On the other hand, persons with pathological throat conditions other than clinical diphtheria, particularly those with diseased tonsils, seem to furnish a somewhat more favorable field for the lodgment and growth of the diphtheria bacillus than that afforded by persons with normal throats."

7. Regarding the relation of the 160 carriers investigated by us to the occurrence of clinical diphtheria either in themselves or in their associates:

(a) Only 11 gave a history of having had diphtheria, but none more recently than three years previous to the time of our study.

* Guthrie, C. G., Gelien, J., and Moss, W. L.: Diphtheria Bacillus Carriers: Second Communication, Johns Hopkins Hosp. Bull., 1920, XXXI, 388.

(b) None of the carriers subsequently developed diphtheria during the period that they were under observation.

(c) Fourteen gave a history of exposure to diphtheria at a period varying from 1 to 12 years previous to the time cultures were made.

(d) A painstaking investigation revealed no case of diphtheria among any of the associates of the 160 carriers.

As a result of these findings we felt justified in emphasizing certain points as follows:

1. The wide prevalence of diphtheria bacillus carriers.

2. The great preponderance of non-virulent bacillus carriers over virulent bacillus carriers.

3. The inability of non-virulent diphtheria bacilli to produce diphtheria.

4. The harmless nature of non-virulent bacillus carriers.

5. The injustice of interfering with the liberties of carriers of non-virulent diphtheria bacilli by quarantine or other measures.

6. The erroneous nature of information concerning the part played by carriers in the spread of diphtheria unless consideration is given to the nature of the organisms harbored by them.

Certain additional information obtained from our study of these children but not embodied in the original report, illustrated in a striking manner one phase of the diphtheria carrier problem, and forms the basis of the present paper.

On looking over the list of 85 carriers detected in the first 800 cultures, some very interesting facts were noted. First, a very large number of these carriers—26, or 30.59 per cent—came from the same street address; second, among the 50 carriers selected at random for repeated examination, 22, or 44 per cent, came from this address; third, among the 81 children from this address, who were examined, 32.09 per cent gave positive cultures. This state of affairs seemed to demand further investigation, which showed that the street number in question was that of an orphan asylum. The information obtained concerning this institution from the superintendent who has had charge of the asylum since 1894 was as follows: The asylum was founded in 1863 and has occupied its present location since 1874. White children only are admitted, but no distinction is made as to nationality or religion. Beyond this the only requirements are (1) that the child is an orphan, (2) is from 3 to 10 years of age, (3) is pronounced sound by the regular medical examiner of the institution and (4) that the financial condition is such as to require aid. At the time our cultures were taken there were 123 inmates, exclusive of the executive staff and attendants, 57 boys between the ages of three and 13 years, and 66 girls ranging in age from three to 17 years. Eighty-five, or 69.17 per cent, of these 123 children attended the public school in which our cultures were taken and 81 of them were included in the first examination of our series and so came under subsequent observation. Of these 81 children, 45 were boys from five to 13 years of age and 36 were girls from six to 14 years of age. Twenty-two of them chanced to be among the 50 carriers selected for repeated examination and thus were recultured at intervals of two weeks over a period of three months. At the final examination practically all of the original 800 children were recultured. Seventy-nine of the 81 children previously studied from the asylum had cultures taken at this time. As was mentioned before, observations on the condition of the throat were also made at each examination and the temperature taken at each examination except the first. The detailed results obtained have been analyzed and are here presented briefly, followed by such comment as seems warranted.

1. *Incidence of Positive Cultures.*—A total of 270 cultures was taken from the 81 inmates of the asylum and 56 positive cultures were obtained; in other words, 20.70 per cent of the cultures taken showed diphtheria bacilli.

Cultures	positive	at 1st	examination	of 81	children	– 26	(32.09 per cent)
"	"	" 2d	"	" 22	"	= 13	(59.09 " ")
"	"	" 3d	"	" 22	"	7	(31.8. " ")
"	"	" 4th	"	" 22	"	– 1	(4.54 " ")
"	"	" 5th	"	" 22	"	3	(13.63 " ")
"	"	" 6th	"	" 22	"	– 2	(9.09 " ")
"	"	" 7th	"	" 79	"	– 4	(5.06 " ")

Total cultures 270. Positive 56 (20.70 per cent)

Thirty, or 37 per cent, of the 81 children, at some time showed a positive culture. A total of 168 cultures was taken from these 30 individuals and of these cultures 56, or 33.21 per cent, were positive.

Individuals	Times cultured	Total cultures	Positive cultures
1	1	1	1
7	2	14	7
1	6	6	3
21	7	147	45
30		168	56

Of the 21 carriers cultured seven times, 8 were positive at only the first examination; 8 were positive twice; 1, three times; 3, four times; 1, six times; and none showed a positive culture at all of the seven examinations.

We failed to find diphtheria organisms in the cultures from 51 of the 81 inmates of the asylum examined but it should be pointed out that each of these 51 children had only two examinations, three months apart.

We were unable to discover any relation between the age or sex of the children and the occurrence of positive cultures.

2. *Character of the Organisms Present in the Positive Cultures.*—This has been dealt with at some length in our earlier report to which reference has been made. In this place it will be sufficient to say that organisms were isolated from 48 of the 56 positive cultures encountered, and these pure strains examined as to morphology, staining characteristics and cultural reactions. The results showed these strains to be typical diphtheria bacilli, not "pseudo-diphtheria bacilli," nor diphtheroids.

Cultures from 26 of the 30 carriers were tested for virulence. From a number of these children who repeatedly showed positive cultures, pure strains were isolated from the successive

positive cultures and virulence tests made upon each of the pure strains, as follows:

1 strain tested from each of	15 carriers	= 15 strains
2 strains " " " "	7 "	= 14 "
3 " " " " "	2 "	= 6 "
4 " " "	1 carrier	= 4 "
5 " " "	1 "	= 5 "
	26 carriers	44 strains

Unfortunately four strains which had been isolated were lost before this test could be made and from 8 of the positive cultures no isolation was made. We are thus unable to say whether the strains of diphtheria bacilli in these 12 cultures were toxin producers or not, and mere speculation on this point is unprofitable. Concerning the 44 strains from 26 carriers which were tested, however, there is no doubt; all of these strains were avirulent.

3. *Occurrence of Pathological Throat Conditions.*—As has been mentioned, the throats of these children were examined and the occurrence of outspoken pathological conditions such as pharyngitis and enlarged or diseased tonsils was noted each time that cultures were taken. Of the 81 children from the asylum who were included in our study, 35, or 43.20 per cent, at some time showed a throat condition regarded as definitely pathological. Of these 35 children who at some time exhibited a diseased condition of the throat, fifteen were examined twice, one was examined six times, and 19 were examined 7 times, making in all 169 examinations. At the 169 examinations on the 35 children, a pathological condition of the throat was noted 78 times, as follows: 17 children showed it but once; 6 showed it twice; 5, three times; 3, four times; 3, five times; and one individual showed it on seven successive examinations. Of the 81 children studied, 46 or 56.79 per cent did not show a pathological throat condition but it should be pointed out that only two of these individuals were examined 7 times; 43 of them had but 2 examinations and one child was examined only once, thus making 101 examinations on these 46 children as contrasted with 169 examinations on the 35 children who at some time showed pathological throat conditions. It is quite probable that more frequent examinations would have materially increased the number of children discovered to have diseased throats. It should be stated that in no instance was a diphtheritic false membrane or pathological membrane formation of any sort observed.

4. *Relation of Pathological Throat Conditions to the Carrier State.*—This matter also has been considered in the earlier report and the impression which we have gained has been expressed in the *résumé* at the beginning of this paper. This impression has not been modified by the findings in this particular group of children. The occurrence of pathological throat conditions and their association with positive throat cultures is presented in this place for a reason which will later be apparent.

The number of children in whom positive cultures and pathological throats were found at the same examination was 14, which is 46.66 per cent of the 30 individuals with positive cultures and 40 per cent of the 35 individuals with pathological throats. Ninety-two cultures were made from these 14 children and of these 35, or 36.95 per cent, were positive. Of these 35 positive cultures, 23, or 65.71 per cent, were obtained from throats noted as pathological at the time the culture was taken. Of the 35 children who at some time showed a pathological throat 22, or 62.85 per cent, at some time showed a positive culture. From these 35 children 169 cultures were made and 45, or 26.62 per cent, proved to be positive. As mentioned above, 22 children who at some time showed pathological throats, at some time were found to be harboring diphtheria bacilli; from these children 143 cultures were taken and 45, or 31.46 per cent of the cultures were positive.

Positive cultures were found in only 8 of the 46 children who at no examination showed a pathological throat condition. From these 8 children 25 cultures were taken and 11, or 44 per cent of the cultures, were positive. In all, 101 cultures were taken from children not noted as having diseased throats and 11, or 10.89 per cent, were positive.

Thirteen of the 35 children with pathological throats had negative cultures, 37.14 per cent. From these 13 children 26 cultures were taken.

Thirty-eight, or 44.44 per cent of the total of 81 children examined from the institution, showed neither pathological throat nor positive culture. From these 38 children 76 cultures were taken.

5. *Temperature Observations.*—Many of the children had an elevation of temperature above 99° F. and in one child it was 100.6° F. although all were attending school at the time our observations were made. These elevations occurred in children showing positive cultures and in those whose cultures were negative; in children with pathological throats and in those whose throats seemed normal; and in children having the various combinations of cultural result and condition of the throat. We did not gain the impression that the carrier state was in itself sufficient to cause an elevation of temperature, although not infrequently associated with such elevations. The condition of the throat noted in many of the children would offer a plausible explanation for an elevation of temperature, but the slight fever present in those with normal throats is not to be explained in this way, nor is it necessary for our purpose to search for the underlying cause in these cases. It is merely desired to emphasize the point that many of the children with positive cultures had also elevations of temperature and pathological throat conditions.

7. *Inmates of the Same Institution Examined the Previous Year (1911).*—During an investigation of diphtheria bacillus carriers in the previous year (1911) *, cultures were taken from 200 children attending the same public school from which the material for our later report was secured. Included among these 200 children were 17 from the orphan asylum in question, about 14 per cent of the inmates at that time. Twenty-one cultures were made from the 17 children and only 1 was

* Moss, W. L., Guthrie, C. G., and Gelien, J.: Diphtheria Bacillus Carriers, Trans. XV Internat. Cong. on Hyg. and Dem., 1912, IV, 156.

found positive. In the course of the present inquiry, 8 of the same children who had been examined during the previous year came under observation and the number of cultures taken from them was 21. None of these 8 children had shown a positive culture in 1911, but 2 of them proved to be carriers when examined in 1912.

8. *Relation of the Carriers to the Occurrence of Clinical Diphtheria.*—Subsequent investigation of the 30 carriers from this institution found in the course of our study of 800 children from one of the Public Schools of Baltimore showed that a history of diphtheria previous to the time of our examination could be obtained from only three individuals. Concerning one child a history was obtained of diphtheria "when a baby"; regarding another, a history of diphtheria "when a small child"; the third had had diphtheria five years before the time that our cultures were taken. In each of these three instances, the disease had occurred before the child became an inmate of the asylum.

None of the 30 carriers developed diphtheria during the period covered by our study, from three to four months. None gave a history of previous exposure to diphtheria. We were unable to discover any cases of clinical diphtheria developing among the associates of these carriers. Six of the 30 carriers gave a history of sore throat during the year, but all had recovered without the aid of a physician.

Perhaps the most interesting information with regard to the relation of these carriers to the community was obtained from the superintendent of the asylum, who was interrogated in 1914 and again in November, 1920. This man has been in charge of the institution since 1894 and his records show that during the 26 years of his incumbency there have been only five cases of diphtheria among the inmates of the asylum. These five cases had occurred in the form of a small outbreak in 1897, 15 years before the time of our investigation. No cases had occurred since that time; on this point he was quite definite.

The question naturally arises as to why we did not investigate the remaining 42 inmates of this interesting orphan asylum and it must be confessed that the explanation, which then seemed adequate, now appears distinctly feeble. Although we had the sanction of the Health Department for our investigations in the public schools we had no authority to invade private institutions. The superintendent of the orphanage, who regarded us with open suspicion, did not take kindly to the idea when casually approached on this subject and no pressure was brought to bear on him, as we were particularly anxious not to call attention to the carrier situation existing in the asylum at that time. Our discovery of this situation was merely incidental in the course of a rather extensive investigation of carriers in which we were engaged and which occupied us fully, so the matter was not pushed.

Summary.—The high incidence of *B. diphtheriæ* in the throats of children from this institution was very striking, as 37 per cent of the 81 inmates examined at some time showed positive cultures, and 20.7 per cent of the total 270 cultures taken contained diphtheria bacilli. There is no reason to suppose that the incidence differed among the other inmates of the asylum, but if the 42 children not examined had all shown negative cultures, there would still have been 24.39 per cent of carriers in the entire institution, a remarkably high figure. Owing to conditions not understood, the ordinary percentage of carriers was in this instance greatly exceeded; this, in turn, served to increase markedly the percentage of carriers found among 800 children from the public school attended by the inmates of this asylum. The incidence of carriers was also much higher than that revealed by the examination of 21 cultures from 17 inmates of the asylum during the previous year, when only one child was found to have diphtheria bacilli in the throat. During the period covered by our observations there was a marked fall in the incidence of carriers; at the first examination 26, or 32.09 per cent of 81 children, had positive cultures; three months later only 4, or 5.06 per cent of the 79 children examined, were found to be harboring diphtheria bacilli in their throats. There was a progressive decrease also in the number of positive cultures obtained at successive examinations of the 22 carriers included by chance in the list selected for repeated study (22, 13, 7, 1, 3, 2, 0). No relation could be discovered between the age or sex of the children and the occurrence of positive cultures.

The identity of the organisms found in the throats was not a matter of surmise, as diphtheria bacilli were isolated from 48 of the 56 positive cultures encountered and these pure strains subjected to careful tinctorial and cultural tests. All proved to be typical in their morphology, staining characteristics and cultural reactions.

Virulence tests were carried out on 44 of the 56 positive cultures encountered, representing pure strains from 26 of the 30 carriers found in this institution. All of the strains were non-virulent.

Many of the children (43.2 per cent) showed throats which were regarded as definitely pathological in appearance and of these children 62.85 per cent had positive cultures. Among the children whose throats seemed normal, only 17.39 per cent had positive cultures. The percentage of positive cultures was also higher among the children with pathological throats (26.62 per cent) than among those with normal throats (10.89 per cent). None of the throats examined showed a diphtheritic membrane or pathological membrane formation of any sort.

Temperature elevations above 99° F. were frequently noted among the inmates of the asylum examined. Such elevations occurred among the children with normal-looking throats whose cultures were positive and among those whose cultures were negative; they occurred in children with evidently pathological throats who showed negative cultures, but the point of interest to us was that many of the carriers with diseased throats also showed a slight fever.

Only 3 of the 30 carriers discovered among the inmates of the institution had previously had diphtheria, none less than five years before the date of our examination, and none since admission to the asylum. None of the children gave a history of previous exposure to diphtheria. A thorough investigation did

not reveal a single case of diphtheria developing among any of the associates of these carriers. There had not been a case of diphtheria in the asylum for 15 years prior to our study; there has not been a case in the eight years which have elapsed since that time.

Extensive comment on these findings seems unnecessary; it will suffice to correlate some of the points brought out by our study and indicate their practical bearing on the problem of diphtheria bacillus carriers. It has been shown that 30 diphtheria bacillus carriers were found on examination of only two-thirds of inmates of the asylum; that pathological throat conditions were common and were frequently found among the diphtheria carriers; that slight fever was also common among the children and occurred among the bacillus carriers having pathological throat conditions. The discovery of a positive culture in a child with an evidently pathological throat and an elevation of temperature might readily suggest the diagnosis of diphtheria and, indeed, many diagnoses are made on no more evidence than this. It should be recalled, however, that in none of these pathological throats was there observed a false membrane or new membrane formation of any sort. Moreover, the 44 cultures tested were all avirulent and therefore presumably incapable of producing any lesion whatever, much less clinical diphtheria. Finally and most important, there is the practical result. No one but ourselves knew of the carriers in the orphanage, consequently the lives of these children were not interfered with in any way and the daily routine of the institution remained undisturbed. The carriers themselves did not develop diphtheria; so far as we could learn their associates did not develop diphtheria; to the best of our knowledge there has been no diphtheria in the asylum for 23 years.

Nevertheless, the possibilities of trouble for the institution at this time were tremendous. Had a case of diphtheria occurred in the public school attended by these children some of them might have had throat cultures taken as contact suspects, and this in turn might have led to investigation of the orphanage. There the apparently alarming state of affairs outlined above would have been discovered, at least in part. Many of these harmless carriers of non-virulent organisms would probably have been regarded as cases of diphtheria, on the basis of a pathological throat, an elevation of temperature and a positive culture. The establishment of a flourishing pseudo-epidemic only awaited the arrival of some well-meaning enthusiast. The institution would have been placarded at once, the children excluded from school, and many of them needlessly subjected to the discomfort of injections of antitoxin for curative or prophylactic purposes. At the end of 12 weeks, as we have seen, the incidence of positive cultures had dropped from 32.09 per cent to 5.06 per cent; this might plausibly have been attributed to the antitoxin used and the measures employed for isolation of the patients. All of these things might very readily have occurred in this orphan asylum; similar occurrences probably take place very frequently.

Conclusions

1. The carrier of avirulent diphtheria bacilli is not a menace to the community.

2. A positive throat culture, an elevation of temperature and a pathological throat condition without definite membrane formation are insufficient evidence on which to base a diagnosis of diphtheria with entire certainty.

3. Virulence tests are necessary to avoid inflicting needless hardships on carriers of avirulent diphtheria bacilli.

STUDIES WITH LECITHIN AND CHOLESTERIN IN RELATION TO THE ANTIHEMOLYTIC PROPERTY OF HUMAN SERUM

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It has been well established that human serum has power to inhibit hemolysis by many different agents,[1] among others soluble soaps and fatty acids, substances occurring in the blood under normal conditions. It is probable that because of this power of the serum the hemolytic substances present in the blood do not cause destruction of the red blood cells in the body. This consideration is of interest since it has been shown[2] that the serum from patients with anemia, as compared with that from normal persons and patients with no anemia, exhibits a diminution in protective power against hemolysis of guinea-pig corpuscles by sodium oleate. The diminution in the protective power of the serum against this hemolytic agent is much more pronounced, both in degree and the regularity with which it is found, in the so-called hemolytic anemias, Addisonian anemia and hemolytic icterus, and in conditions in which the liver or spleen is extensively or prominently involved in the disease process. The experiments from which this observation was drawn, although affording no proof for the following suggestion, at least raised the possibility that the hemolysis in the hemolytic anemias may be due to hemolytic agents normally in the blood, enabled to act as hemolysins because of the diminution in the property of the serum which normally inhibits such activity. For these reasons it appeared important to study closely the substance or substances in the serum responsible for the protective power exhibited by serum against hemolysis.

Among the substances in the serum that might be considered in some way related to hemolysis or the inhibition of hemolysis, lecithin and cholesterin were selected for study first. In studies of hemolysis much attention has been given to the activity of both lecithin and cholesterin and it has been shown that they are of great importance in this connection.

The facts so far demonstrated, however, do not permit one to postulate clearly just what their action in relation to hemolysis may be in the body. Thus, lecithin itself is a hemolytic agent, and hemolysis by lecithin may be inhibited by cholesterin. Cobra venom is activated by lecithin with the production of a new substance in itself hemolytic, the so-called cobra-lecithid. This cobra-lecithid hemolysis is inhibited by cholesterin, the inhibitory action being due to the interaction of lecithin and cholesterin, possibly the solubility of one in the other.[3] Blood serum may activate cobra venom as lecithin does, and it may also inhibit the activity of cobra-lecithid as cholesterin may do. These properties of blood serum probably depend upon its content of lecithin and cholesterin. In studies with the Much-Holtzmann Psychoreaction, Bauer[4] suggests that increases in the protective power of serum against cobra venom hemolysis probably are due to the paucity of activating lipoids, perhaps lecithin, rather than to an increase in inhibiting substances such as cholesterin. In contradistinction to its action in cobra venom hemolysis where lecithin acts as an activator, with some hemolytic agents it acts rather as a protective substance and tends to inhibit hemolysis caused by them. Thus Arrhenius[5] reported that, if red blood cells are exposed to a lecithin solution beforehand, their resistance to hemolysis by saponin is markedly increased. This same investigator made the observation also that red blood cells so treated exhibit a decrease in resistance to hemolysis by acids, but no alteration in resistance to hemolysis by alkalies. In addition to this, Bayer[6] states that lecithin protects slightly against hemolysis by bile salts. Cholesterin, as compared with lecithin, is not in itself a hemolytic agent and has not as yet been shown to combine with another substance to form an active hemolysin such as occurs with lecithin and cobra venom. Cholesterin does, however, inhibit hemolysis by lecithin and cobra-lecithid. Ransom[7] has shown that cholesterin prevents the hemolysis caused by saponin. It is interesting that lecithin also inhibits hemolysis by this substance;[5] but hemolysis by another substance, bile salts, which is retarded by lecithin, is not influenced by cholesterin.[6] According to Liebermann and Fenyvessy,[8] if cholesterin is boiled with sodium oleate, the hemolytic power of the sodium oleate will be destroyed. In this instance another kind of interaction between lecithin, cholesterin and hemolytic agents is presented, namely, hemolysis inhibited by cholesterin but not by lecithin, for, as brought out in some of the experiments to be reported below, lecithin will not inhibit hemolysis by sodium oleate. From this brief discussion it will be seen that several kinds of relation between lecithin, cholesterin and hemolytic agents have been demonstrated. Thus lecithin is itself a hemolytic agent, and hemolysis by it is inhibited by cholesterin. Lecithin combines with cobra venom to form an active hemolysin, the activity of which is prevented by cholesterin. Lecithin inhibits hemolysis by several substances, hemolysis by one of which, saponin, is also inhibited by cholesterin; but with another, bile salts, no inhibitory power is exhibited by cholesterin. And cholesterin may, under certain conditions, inhibit the action of sodium oleate against which lecithin is inert as a protective agent.

In view of the points discussed above it is of interest that Bloor and Knudson[9] reported a hypocholesterinemia in pernicious anemia, and Gorham and Myers[10] in all anemias of pernicious type and in cachexias of malignancy. Of equal interest is the observation of Denis[11] that after splenectomy in one case of pernicious anemia, one of family jaundice, one of "splenic anemia," and one of Banti's disease, both the lecithin and cholesterin were increased in the whole blood and plasma, and that with improvement in the blood picture and clinical condition the cholesterin percentage rose.

Thus, although the rôle of lecithin and cholesterin in any hemolytic process which may occur in the body may not be stated definitely, there are several facts established which, when considered together, are suggestive as regards the protective property of the serum which may be of importance in relation to anemia and point to these substances for study in this connection. These facts are: first, the diminution in the protective power of serum from cases of so-called hemolytic anemias against hemolysis of guinea-pig corpuscles by sodium oleate; second, the protective power manifested by cholesterin against the action of some hemolysins; and third, the diminution of blood cholesterin in anemias of the pernicious type. If any close interrelation between these facts could be demonstrated it might be possible to say with some assurance upon what substance the antihemolytic property of blood serum depends. More particularly, light might be shed on the nature of the antihemolytic substance in the serum which may be of importance in hemolytic anemias. Accordingly, the following experiments were undertaken to correlate, if possible, the lowered cholesterin in the blood and the diminished protective power of the serum against hemolysis by sodium oleate in the so-called hemolytic anemias. Because of the intimate relationship existing between lecithin and cholesterin in the body and the complexity of their action in hemolytic processes, it seemed probable that if one of them were concerned in any hemolytic process the other would be involved also, and for this reason parallel studies were carried out with lecithin. As a control observation all experiments were done not only with sodium oleate, a hemolytic agent probably occurring normally in the body, but also with saponin which is a hemolysin foreign to the body tissues.

The experiments undertaken to study the points outlined above were as follows:

(1) Quantitative measurements were made of the protective power against hemolysis by sodium oleate and saponin of serum extracted with petroleum ether, and of the petroleum ether extract of serum.

(2) The activities in relation to hemolysis of lecithin and cholesterin, alone and in various combinations, and under different conditions, were studied by the same methods.

(3) The influence of both lecithin and cholesterin, alone and together under various conditions, on the protective power of serum from normal people and from cases of pernicious anemia was studied by quantitative measurements of protective power against hemolysis.

The method of making the quantitative measurements was the same as that outlined in an earlier paper on the study of the protective power of human serum against hemolysis by sodium oleate. A series of test-tubes, each containing 2 c. c. of a different dilution of hemolytic agent, was set up and to each tube of the series 0.25 c. c. of the substance to be tested for protective power was added. This was put in the water-bath at 37° C. for one-half hour, allowed to cool one-half hour, and then 0.25 c. c. of a 0.75 per cent suspension of guinea-pig cells was added to each tube. This preparation was incubated at 37° C. in the water-bath for two hours, allowed to sediment in the ice-box for an hour and then a reading of hemolysis in each tube was made. The dilutions of sodium oleate commonly used were from 1-45,000 to 1-90,000 in steps of 5000, thus: 1-45,000; 1-50,000; 1-55,000; 1-60,-000; 1-65,000, and so on. With this method it was found, as reported in an earlier paper,[2] that serum from normal persons and patients with no anemia regularly protected guinea-pig corpuscles from hemolysis by sodium oleate up to strengths of 1-55,000 or higher, but that serum from patients with a hemolytic anemia or with an anemia and involvement of the liver or spleen rarely protected against strengths of sodium oleate beyond 1-75,000 and often only against solutions of hemolytic agent even weaker than this. Accordingly, in these experiments as in the earlier ones with serum the series of tubes carried dilutions of sodium oleate from 1-45,000 to 1-90,000 in steps of 5000. The technique of handling and the precautions to be taken in the preparation of the dilutions of sodium oleate, in the preparation of the guinea-pig cell suspension, in the cleaning of glassware, the method of titration, and the controls run, is presented in our earlier paper to which the reader is referred for details. The preparation of the materials tested for protected power will be given in this paper as experiments with them are taken up. When saponin was used as the hemolytic agent, it was found that normal serum protected guinea-pig corpuscles up to strengths of 1-18,000 to 1-20,000, and, as will be reported later in detail, the serum from those cases in which striking reduction in protective power against hemolysis by sodium oleate was found, protected against hemolysis by saponin only up to strengths of 1-26,000, 1-28,000 and 1-30,000. Therefore, with this weaker hemolytic agent saponin, as compared with sodium oleate, the series of tubes carried dilutions of 1-18,000 to 1-34,000 in steps of 2000. Other than the greater strengths of solutions used, the technique with the saponin was entirely the same as that previously detailed for sodium oleate.

In the technique outlined in our earlier paper, when serum was used as the protecting agent, it was always inactivated on the water-bath at 56° C. before being used. In some experiments presented in this paper, serum was used without previous inactivation and when so used this is stated. When no mention is made about this point the serum used had been inactivated.

I. Studies With Cholesterin Alone

All the cholesterin used in these experiments was extracted from gall-stones and was recrystallized three times. Three different methods of using it were employed:

(a) Cholesterin suspended in sterile normal salt solution was tested to see if it alone had any protective power against hemolysis, if it was inert, or if when so used it increased the hemolytic power of the hemolytic agent.

(b) Cholesterin was added to the hemolytic agents to determine what action it had on the hemolytic power exhibited by them.

(c) Cholesterin was added to serum to see if it increased or diminished the protective power of the serum.

(a) To determine the activity of cholesterin suspended in normal salt solution the cholesterin preparation was used in exactly the same way as serum in the titration for protective power against hemolysis as already described for serum. In the preparation of the cholesterin emulsion in salt solution, 10 milligrams of cholesterin were dissolved in 3 c. c. of a mixture of absolute alcohol 3 parts, ether 1 part. This was evaporated to 0.5 c. c. volume and then poured slowly and with constant stirring into 9 c. c. of sterile normal salt solution and the mixture brought up to 10 c. c. by the addition of more salt solution. A cloudy, milky fluid resulted. After standing overnight a white flocculent precipitate separated out, but when shaken the mixture took on the same milky appearance as when first made. For the test, 0.25 c. c. of this mixture was added to each tube of the hemolytic series just as serum diluted 1-20 was added to each tube in tests to measure quantitatively the protective power against hemolysis exhibited by a given serum. According to the figures of Bloor and Knudson[9] normal blood contains 175 to 200 milligrams of cholesterin per 100 c. c. If these values are correct, the 0.25 c. c. serum diluted 1-20, added to each tube of the hemolytic series, contained 0.025 milligrams or less of cholesterin. In the cholesterin preparation used in this titration the 0.25 c. c. of it added to each tube of the hemolytic series contained 0.25 milligrams of cholesterin. This is just ten times as much cholesterin as is contained in the serum added when tests are made to determine quantitatively its protective power. A parallel titration of the protective power of normal serum was carried out and at the same time the hemolytic series was set up with guinea-pig corpuscles unprotected by serum. In this last test the customary total volume in each tube of the series, 2.5 c. c., was made up by the addition of 0.25 c. c. of sterile normal salt solution instead of serum. By comparison with the titration of normal serum it was possible to determine whether the cholesterin preparation showed as much protection as the serum, and by comparison with the results of the test with unprotected guinea-pig cells, whether any protection was afforded by the cholesterin suspension, whether it was inert, or whether by its presence it increased the hemolytic power of the hemolytic agent.

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-55,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-160,000.

Cholesterin suspension in salt solution—Partial hemolysis up to and including dilution 1-170,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-140,000.

Cholesterin suspension in salt solution—Partial hemolysis up to and including dilution 1-40,000.

These experiments demonstrate that cholesterin suspended in normal salt solution affords no protection against hemolysis of guinea-pig corpuscles by sodium oleate. The slightly increased hemolysis seen in the titrations in which cholesterin in salt was used might have been due to the alcohol and ether in which the cholesterin was originally dissolved, a slight amount of which no doubt was introduced with the cholesterin suspension in salt solution. Cholesterin thus suspended in salt solution has marked protective power against hemolysis by saponin, but even in the relatively large amounts used does not protect as powerfully as normal serum.

(b) To determine what action cholesterin had when added to sodium oleate and saponin, cholesterin crystals were added directly to each and the usual titration of a normal serum for protective power was done with the hemolytic agents thus treated. Of a 1-1000 solution of sodium oleate and of saponin 100 c. c. were prepared as usual for the ordinary titration of protective power of serum and each was divided into two equal portions. To one of the 50 c. c. portions of sodium oleate and to a similar one of the saponin preparations 25 milligrams of cholesterin were added. The four preparations resulting, one of sodium oleate 1-1000, one of sodium oleate 1-1000 plus 25 milligrams of cholesterin, one of saponin 1-1000, and one of saponin 1-1000 plus 25 milligrams of cholesterin were kept in the incubator overnight and the further dilutions of hemolytic agent made the next morning as usual just before the test was to be done. In the morning the preparations to which cholesterin crystals had been added showed a white flocculent precipitate which diffused throughout the mixture with shaking. The only difference between these and the preparation of an ordinary titration for protective power against hemolysis was that there were not only hemolytic series of sodium oleate and saponin but also of sodium oleate and saponin to which cholesterin had been added the night before.

Titration of normal serum with each of these resulted as follows:

Sodium oleate:

Ordinary preparation—Partial hemolysis up to and including dilution 1-50,000.

Cholesterinized sodium oleate—Partial hemolysis up to and including dilution 1-45,000.

Saponin:

Ordinary preparation—Partial hemolysis up to and including dilution 1-20,000.

Cholesterinized saponin—Partial hemolysis up and including dilution 1-20,000.

These experiments show that cholesterin added as crystals to sodium oleate diminishes slightly its hemolytic power for guinea-pig corpuscles in the presence of normal human serum; and when added to saponin it does not influence its hemolytic power.

(c) To see whether cholesterin added to serum increased or diminished its protective power, 10 milligrams of cholesterin were added to 5 c. c. of serum and this was kept in the incubator overnight. The next day the serum showed no change in appearance and about the same amount of cholesterin crystals as had been added the day before was sedimented on the bottom of the tube. This tube was centrifuged and the serum free from crystals was pipetted off the top. Titrations of the protective power of this serum to which cholesterin crystals had been added, and then removed after a period of incubation, were carried out in the usual way and the results compared with those from the same serum to which no cholesterin had been added were as follows:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-55,000.

Cholesterinized serum—Partial hemolysis up to and including dilution 1-55,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Cholesterinized serum—Partial hemolysis up to and including dilution 1-20,000.

From these results one may conclude that when cholesterin crystals are added directly to serum and later removed, the procedure does not influence its protective power against hemolysis by sodium oleate or saponin.

II. Studies With Lecithin Alone

The lecithin used in these experiments was a specimen supplied by the Digestive Ferments Company of Detroit and each time just before use was taken from the container in which it was received. The properties of lecithin in relation to hemolysis by sodium oleate and saponin were studied by the same methods used for cholesterin.

(a) Lecithin in salt solution was used in a quantitative titration for protective power against hemolysis.

(b) Lecithin was added to the hemolytic agents and the hemolytic power of these was tested.

(c) Lecithin was added to serum and the protective power against hemolysis of the serum thus treated was tested.

(a) When the action of lecithin in salt solution as compared with that of serum was studied, titrations for protective power against hemolysis were carried out in which lecithin in salt solution was added to the hemolytic series in exactly the same

manner as serum when similar studies already described were made with serum. To prepare the lecithin in salt solution 10 milligrams of lecithin were suspended in 100 c. c. of sterile normal salt solution and this 1-10,000 suspension was allowed to stand in the ice-box overnight. A slightly cloudy, yellowish fluid resulted. Of this suspension 0.25 c. c. was added to each tube instead of the serum diluted 1-20 as in tests for the protective power of serum, so that the dilution of lecithin in the final preparation in each tube of the hemolytic series was 1-100,000 (0.25 of a 1-10,000 lecithin suspension was put in each tube, the final volume of which was regularly 2.5 c. c. in the titration as set up). As with cholesterin, a parallel titration of the protective power of normal serum was carried out and at the same time a hemolytic series with guinea-pig corpuscles unprotected by serum was set up. The following results were obtained:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-55,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-180,000.

Lecithin preparation—Complete hemolysis up to and including dilution 1-180,000 almost immediately: that is, with less than half an hour's incubation. (In the usual test, hemolysis was recorded only after two hours' incubation and one hour in the ice-box.)

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-140,000.

Lecithin preparation—Partial hemolysis up to and including dilution 1-90,000.

The results of these experiments show that lecithin suspended in salt solution, when tested with a hemolytic series, does not protect against hemolysis by, but increases the hemolytic power of, sodium oleate. The same preparation does protect somewhat against hemolysis by saponin, but in the relatively large amounts used does not afford as much protection against saponin hemolysis as does normal serum.

(b) To determine what action lecithin exerts, when added directly to the hemolytic agents, the same methods were used as described above when similar tests were made with cholesterin. Twenty milligrams of lecithin were added to 50 c. c. of a 1-1000 sodium oleate solution and the same amount to 50 c. c. of a 1-1000 saponin solution. These preparations were allowed to stand overnight in the incubator and in the morning there was a slight yellowish-white precipitate which readily diffused through the mixtures with shaking, giving fluids of slightly cloudy, yellowish appearance. The results of the titrations of normal serum with hemolytic agents thus treated, compared with titrations of the same serum with the usual hemolytic agents, were as follows:

Sodium oleate:

Ordinary preparation—Partial hemolysis up to and including dilution 1-50,000.

Lecithinized sodium oleate—Partial hemolysis up to and including dilution 1-60,000.

Saponin:

Ordinary preparation—Partial hemolysis up to and including dilution 1-20,000.

Lecithinized saponin—Partial hemolysis up to and including dilution 1-20,000.

These experiments show that lecithin added to sodium oleate even in the minute quantities used here increases somewhat its hemolytic power for guinea-pig corpuscles in the presence of normal human serum, but exerts no influence on the hemolytic power exhibited by saponin.

(c) To see whether lecithin added to serum influences its protective power against hemolysis, the same method of testing was again used as described above when similar tests were made with cholesterin; but instead of adding the lecithin directly to serum as was done with cholesterin, it was added in the salt solutions used to dilute the serum 1-20. Ten milligrams of lecithin were suspended in 100 c. c. of sterile normal salt solution and this lecithinized salt solution was used to make the 1-20 dilution of serum instead of sterile normal salt solution ordinarily employed. Of this preparation, 0.25 c. c. was added to each tube of the hemolytic series giving a concentration of lecithin of 1-105,000 in the final preparation. The titrations carried out with this lecithinized serum and with the same serum to which no lecithin had been added resulted as follows:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-55,000.

Lecithinized serum—Partial hemolysis up to and including dilution 1-170,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Lecithinized serum—Partial hemolysis up to and including dilution 1-26,000.

These experiments show that lecithin added to serum decreases the protective power against sodium oleate markedly; or what is probably more nearly the correct interpretation, it increases the hemolytic power of the system by adding the hemolytic power of lecithin to that of the sodium oleate.*

* When one compares the results of the experiments where lecithin was added (1) to the sodium oleate and (2) to the serum, an apparent discrepancy is seen. The difference is explained by the fact that in the former, where hemolysis was increased slightly, the amount of lecithin present was much less than in the latter, where hemolysis was increased markedly. In the experiment in which lecithin was added to the sodium oleate, it was added to the 1-1000 stock solution and, therefore, when the greater dilutions were made from this, each tube of the hemolytic series contained decreasing amounts of lecithin as follows:

Sodium Oleate	Lecithin
1–50,000	1–125,000
1–55,000	1–137,500
1–60,000	1–150,000
1–65,000	1–162,500
Etc.	

In the experiment in which lecithin was added to the serum, however, the lecithin was in a concentration of 1–105,000 in each tube.

Also it appears that the protective power of the serum against saponin hemolysis is diminished.

III. Studies With Lecithin and Cholesterin Together

Cholesterin is not soluble in salt solution. It seemed possible that if cholesterin were suspended in the proper vehicle, it might protect against hemolysis by sodium oleate as it does against saponin when emulsified in salt solution. Lecithin was chosen for this purpose, since it is a solvent for cholesterin and moreover one which is widely distributed throughout the body and therefore presumably available for this purpose *in vivo*. In these experiments the same lecithin and cholesterin were used as in those detailed above. Two methods were employed:

(a) Lecithin suspended in salt solution and containing as much cholesterin as would go into solution was used in a quantitative titration for protective power against hemolysis.

(b) Serum to which lecithin had been added in minimal quantities and then as much cholesterin as would dissolve was tested for protective power against hemolysis.

(a) In making the preparation of both lecithin and cholesterin in normal salt solution to be tested for protective power against hemolysis, 10 milligrams of lecithin were suspended in 100 c. c. of sterile normal salt solution as in the test with lecithin alone, and a knife-point of cholesterin crystals was added. This was allowed to stand in the incubator overnight and the cholesterin crystals remaining were removed by filtration. The filtrate showed a moderately strong positive test for cholesterin, when tested by Liebermann's acetic anhydride-sulphuric acid method. This resulted in a 1-10,000 suspension of lecithin in normal salt solution containing as much cholesterin as would dissolve under these conditions. For this test 0.25 c. c. of this preparation was added to each tube in the hemolytic series as in all other similar titrations discussed above, giving a final concentration of lecithin 1-100,000 in each tube of the hemolytic series. Titrations of normal serum and a hemolytic series with guinea-pig corpuscles unprotected by serum were set up with the same hemolytic agents. The following readings were made:

Sodium oleate:

- Normal serum—Partial hemolysis up to and including dilution 1-55,000.
- Unprotected guinea-pig corpuscles—Partial hemolysis up to and including dilution 1-180,000.
- Lecithin-cholesterin preparation in normal salt solution—Partial hemolysis up to and including dilution 1-180,000 (after one hour's incubation).

Saponin:

- Normal serum—Partial hemolysis up to and including dilution 1-20,000.
- Unprotected guinea-pig corpuscles—Partial hemolysis up to and including dilution 1-140,000.
- Lecithin-cholesterin preparation in normal salt—Partial hemolysis up to and including dilution 1-60,000.

These experiments show that a 1-10,000 suspension of lecithin in normal salt solution containing cholesterin in solution does not afford any protective power against hemolysis by sodium oleate, but that hemolysis is increased. This increase is probably due to the lecithin added, for as shown above, lecithin introduced adds to the hemolytic power of the system. With lecithin-cholesterin preparation in normal salt solution, however, the increase in hemolysis is not quite so much as when lecithin alone is used. The correct interpretation of this probably is that the cholesterin inhibits a part of the added hemolysis due to the lecithin present. From these results it also appears that the lecithin-cholesterin salt preparation exerts a rather marked protective power against hemolysis by saponin, but even in the relatively large amounts employed does not protect nearly as strongly as normal serum.

(b) To test how the addition of both lecithin and cholesterin to normal serum would influence its protective power against hemolysis, one point had to be established first. It has already been shown that when lecithin was added to serum in such quantities that in the final preparation there was a concentration of 1-100,000 of lecithin in each tube of the hemolytic series, the protective power was diminished. The point to be determined was whether or not lecithin could be added to the serum in such small quantities that, when a titration was done, it did not react towards sodium oleate differently than normal serum. A series of tests showed that, if lecithin was added to the serum in such quantities that in the final preparation in each tube of the hemolytic series it was in a concentration of 1-300,000 or less, the results with sodium oleate did not differ from those obtained when normal serum was used.* Cholesterin crystals were added to this 1-30,000 lecithin solution and were allowed to remain in contact with the solution overnight in the incubator. The cholesterin crystals remaining were then removed by centrifugation. The supernatant fluid contained cholesterin in solution as shown by a positive Liebermann's test. This material was used to dilute the serum 1-20 instead of normal salt solution just as in the experiments with lecithin alone, and titrations for protective power against hemolysis of serum thus treated were made. At the same time titrations of normal serum and of serum to which lecithin had been added in the small quantity shown not to influence its reaction with sodium oleate were done. The results obtained were as follows:

Sodium oleate:

- Normal serum—Partial hemolysis up to and including dilution 1-55,000.
- Lecithinized serum—Partial hemolysis up to and including dilution 1-55,000.

* The amount of lecithin which could be added to serum without lowering its protective power for saponin was not determined. The amount of lecithin, which, added to serum, did not increase the hemolysis seen with sodium oleate, did, however, increase the hemolysis with saponin. This is surprising, because lecithin in the absence of serum, protects slightly against saponin hemolysis, whereas with sodium oleate the amount of hemolysis is increased. This finding was the same in several duplicate experiments at different times. No explanation of this apparent paradox has so far presented itself.

Serum to which lecithin and cholesterin had been added—Partial hemolysis up to and including dilution 1-50,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Lecithinized serum—Partial hemolysis up to and including dilution 1-26,000.

Serum to which both lecithin and cholesterin had been added—Partial hemolysis up to and including dilution 1-20,000.

The results obtained in these experiments show that when lecithin, in such small amounts that it does not increase hemolysis with sodium oleate, is treated with cholesterin and this mixture is added to serum, the protective power of the serum against hemolysis by sodium oleate is increased, but only to a slight degree. The same amount of lecithin, which added to serum does not increase the hemolysis seen with sodium oleate, but does increase hemolysis with saponin, when combined with cholesterin, affords some protection against saponin as compared with a solution of lecithin alone of similar strength in normal salt solution. In this case again, the correct interpretation probably is that the cholesterin merely neutralizes the increased hemolysis due to the lecithin present.

IV. Studies With Cases of Pernicious Anemia

In experiments already discussed, lecithin alone, cholesterin alone, and lecithin and cholesterin together were added to normal serum which had been inactivated and the effect on the protective power of the serum against hemolysis by sodium oleate and saponin recorded. Exactly the same technique being employed, similar experiments were carried out with serum from patients with pernicious anemia. The results were the same as those obtained when normal serum was used. Cholesterin added to serum afforded no increased protective power against sodium oleate or saponin. Lecithin in a strength of 1-30,000, and containing as much cholesterin as it would dissolve added to serum gave no increased protection against sodium oleate or saponin.

V. Studies With Serum That Had Not Been Inactivated

All the experiments detailed above were carried out with serum that had been inactivated. It appeared possible that the activity of normal ferments of the blood might be necessary before cholesterin added to serum could act as an antihemolytic agent. Accordingly, cholesterin was added to sera that had not been inactivated, from normal persons and from patients with pernicious anemia of different degrees of severity. Titrations carried out with these sera showed, however, that they did not react differently than inactivated serum to which cholesterin had been added.

VI. Studies With Serum After Extraction With Petroleum Ether

In further attempts to correlate two of the findings in pernicious anemia, the diminished cholesterin in the blood and the lowered protective power against hemolysis by sodium oleate, quantitative estimations of the protective power of serum against hemolysis by sodium oleate and saponin were carried out after an attempt had been made to remove the cholesterin from serum by extraction with petroleum ether. The petroleum ether extract was similarly tested. The petroleum ether used in the extractions was re-distilled at a temperature below 56° C. before use. Five cubic centimeters of serum were extracted vigorously in a continuous extractor for 12 hours at a temperature kept constantly below 56° C. When the petroleum ether used in the extraction was tested for cholesterin by Liebermann's reaction, it gave a strongly positive test. All the cholesterin had not been removed from the serum, however, for when the extracted serum was similarly tested, a trace of cholesterin was found to be present.

(a) The serum thus extracted was heated for two hours on the water-bath at 56° C. to remove all traces of petroleum ether. It was then used in a titration of protective power against hemolysis just as was done with normal serum. The results obtained from this and a titration of normal serum done at the same time were as follows:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-60,000.

Extracted serum—Partial hemolysis up to and including dilution 1-105,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Extracted serum—Partial hemolysis up to and including dilution 1-34,000.

The results of these experiments show that, when serum is extracted with petroleum ether, there is a marked diminution in the protective power against hemolysis by sodium oleate and saponin.

It seemed possible that the increased hemolysis observed, when extracted serum was used to protect against sodium oleate and saponin, might not be due to a reduction in the protective power of the extracted serum but rather to an increase in the hemolytic power of the series because of a trace of petroleum ether, an active hemolytic agent, possibly remaining in the serum when used. The control tube set up with each titration to show that the serum used was not hemolytic in itself was negative in this instance as in all others, indicating that any trace of petroleum ether possibly in the serum was not in sufficient concentration to influence the hemolysis in the test. To control this point further, however, the following experiment was carried out:

Three drops of the same lot of petroleum ether used in the extraction were added to 1 c. c. of serum and a titration of this serum for protective power was made.

This titration resulted as follows:

Normal serum showed with sodium oleate—Partial hemolysis up to and including dilution 1-55,000.

Normal serum plus petroleum ether showed with sodium oleate—Partial hemolysis up to and including dilution 1-60,000.

Furthermore, this serum to which petroleum ether had been added showed a negative control tube, indicating that the serum itself as used had no hemolytic properties.

In view of these findings one is justified in concluding that the greatly increased hemolysis resulting when extracted serum was used was not due to any trace of petroleum ether which might possibly have been present in the serum when titrated.

(b) To study the petroleum ether extract for protective power against hemolysis, the petroleum ether used in the extraction of the serum and shown to contain cholesterin, was evaporated to dryness. The residue was taken up by boiling with successive portions of a mixture of absolute alcohol three parts and ether one part, and the combined portions were evaporated to 1 c. c. Half a cubic centimeter of this was added to 9 c. c. of sterile normal salt solution slowly and with constant stirring, and 0.5 c. c. was added to 9 c. c. of normal salt solution containing lecithin in a strength of 1-30,000. Each portion probably contained then much, if not most, of the cholesterin from 2.5 c. c. of serum, and one of them in addition the amount of lecithin which had been shown to be without influence in itself on the reaction of serum with sodium oleate. For this test 0.25 c. c. of each preparation was added to each tube of the hemolytic series in exactly the same way as serum in the usual test for the protective power of serum. At the same time the customary titration of normal serum and a hemolytic series with guinea-pig corpuscles unprotected by serum were set up for comparison. The following results were obtained:

Sodium oleate:

Normal serum—Partial hemolysis up to and including dilution 1-60,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-180,000.

Petroleum ether extract—Partial hemolysis up to and including dilution 1-180,000.

Petroleum ether extract to which lecithin had been added—Partial hemolysis up to and including dilution 1-180,000.

Saponin:

Normal serum—Partial hemolysis up to and including dilution 1-20,000.

Unprotected guinea-pig cells—Partial hemolysis up to and including dilution 1-140,000.

Petroleum ether extract—Partial hemolysis up to and including dilution 1-40,000.

Petroleum ether extract to which lecithin had been added—Partial hemolysis up to and including dilution 1-24,000.

The results of these titrations demonstrate that the residue from evaporation of the petroleum ether used in extracting serum, when taken up in alcohol-ether and suspended in salt solution has no protective power against hemolysis by sodium oleate either alone or after lecithin had been added. The petroleum ether extract, when similarly treated, has considerable protective power against hemolysis by saponin and this is increased in the presence of lecithin.

In summary, several points of interest have been demonstrated in these experiments.

(1) Cholesterin emulsified in salt solution does not protect against hemolysis by sodium oleate but does protect against hemolysis by saponin. However, when cholesterin, without previous solution in alcohol-ether and suspension in salt solution, is added as crystals to serum, either inactivated or not inactivated, and the crystals removed after a definite period, it does not increase the protective power of the serum against hemolysis by sodium oleate or saponin; and if added as crystals to either of these hemolytic agents, it does not diminish their hemolytic power.

(2) Lecithin suspended in salt solution increases the hemolysis seen with sodium oleate but protects slightly against hemolysis by saponin. In the presence of serum, however, lecithin increases the hemolysis seen with both sodium oleate and saponin.

(3) A relatively strong suspension of lecithin in salt solution and containing cholesterin in solution does not protect against hemolysis by sodium oleate but does afford protection against saponin. When a weak suspension of lecithin in which cholesterin has been dissolved is added to serum, the protective power of the serum against sodium oleate and saponin is not increased.

(4) Cholesterin and lecithin, alone and together, added to serum from cases of pernicious anemia do not react differently in relation to hemolysis by sodium oleate and saponin than when added to normal serum.

(5) Serum, when vigorously extracted by petroleum ether at a temperature constantly below 56° C., loses most of its protective power against hemolysis by sodium oleate and saponin. The residue, after evaporation of the petroleum ether used in the extraction of serum, reacts just as cholesterin in relation to hemolysis by sodium oleate and saponin; *i. e.*, when taken up in an alcohol-ether mixture and emulsified in normal salt solution, or in normal salt solution containing lecithin, it affords no protection against hemolysis by sodium oleate, but does protect against saponin hemolysis.

The results obtained in these experiments indicate that there is much to be learned concerning the part played by cholesterin and lecithin in hemolytic processes and in particular, the hemolytic processes which may occur in the body. They do not permit one to suggest any possible relationship which cholesterin and lecithin may have to the hemolysis occurring in the hemolytic anemias.

Conclusions

(1) The protective power of the serum normally present against hemolysis by sodium oleate and shown to be diminished in hemolytic anemia has not been shown to be directly dependent upon:

(a) Cholesterin alone.

(b) Lecithin alone.

(c) Simple combinations of lecithin and cholesterin as used in these experiments.

(2) The protective power of serum normally present against hemolysis by saponin, also diminished in hemolytic anemias, has not been shown to be wholly dependent upon:

(a) Cholesterin alone.

(b) Lecithin alone.

(c) Simple combinations of lecithin and cholesterin as used in these experiments.

(3) The diminished protective power against hemolysis of the serum in hemolytic anemia has not been explained simply on the basis of the diminution in the total cholesterin of the blood found in these conditions.

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VARIATIONS IN THE BACTERIAL FLORA OF THE UPPER AIR PASSAGES DURING THE COURSE OF COMMON COLDS

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Introduction

Despite the frequency and prevalence of the common cold remarkably little accurate information is available bearing on the exact nature and cause of this malady. Yet this is not surprising, for the very mildness of the disease, as a rule, keeps the patient from seeking medical aid until some grave complication supervenes. Furthermore, even if the physician is consulted, the circumstances are usually not conducive to careful study and observation, since the patient is ambulant, and is not seen under conditions which promote the keeping of careful records. Finally, the mildness of the symptoms makes exact definition of the disease difficult, and the primary cold is often confused with sinus infections, tonsillitis, laryngitis, or other complicating disturbances of the upper air passages.

None the less it seems of the utmost importance that the problem of the common cold be solved. Although one does not die of this disease, the total morbidity is tremendous—few people go through a winter without at least one acute coryza, and the sum total of inconvenience to a community from this cause can hardly be overestimated. In addition, a cold is often the starting point for a variety of more serious and intractable disturbances, such as sinus and middle-ear infections, laryngitis and bronchitis. Furthermore, it seems quite possible that an acute coryza may be related to the development of carrier states involving organisms which may cause even more serious diseases, such as pneumonia and meningitis; in fact the ultimate pathological sequences of the cold may be vastly more extensive than one would at first sight suppose. Finally, were the cause and nature of this malady elucidated much important knowledge on epidemiological matters might be obtained, and the study of other respiratory diseases, such as influenza, might be simplified.

During the past winter we have made certain bacteriological studies on colds which we desire to present, but it seems advisable first to review the literature and to discuss the clinical features of the disease.

Clinical Notes

In the study of any disease various lines of attack may be pursued. If the causal agent be definitely ascertained, special bacteriological or other methods may suffice in selecting cases which comprise one entity, but in the absence of such knowledge more advance may perhaps be made by careful clinical observation of the natural history of the disease, and by attempting to relate it to other conditions generically similar

but specifically different. Thus, in the recent epidemic of influenza, pure bacteriological studies led merely to confusion, whereas clinical observation differentiated the primary disease from its picturesque but unessential complications, and drew a definite analogy between it and certain other diseases of unknown etiology such as measles. It seems worth while, therefore, to present a brief analysis of the disease—acute coryza—from the clinical standpoint.

In uncomplicated cases we recognize two sets of phenomena—the constitutional reaction, and the local disturbances in the upper air passages. Even in mild cases the former are rarely lacking—malaise, chilly sensations, headache or general aching, and slight fever. The local reaction manifests itself by dryness, rawness or burning of the mucous membranes of the nose, pharynx or palate, which is soon followed by some swelling of the parts, as evidenced by nasal occlusion, sneezing and lachrymation, and by more or less watery discharge. In simple cases the constitutional reaction subsides in a few days, and the nasal and pharyngeal discharge, after becoming more abundant and mucoid, disappears. The patient is well, but the mucous membranes may feel raw or dry for days or even a few weeks. In uncomplicated cases there is never any local pharyngeal or tonsillar exudate or any really purulent discharge; the local condition seems to consist simply of a hyperemia and swelling of the mucous membranes. About 20 cases were available for study in the records of The Johns Hopkins Hospital and from these histories the following information was obtained—fever, if present, never lasts more than a few (two or three) days, the leucocytes are diminished, or at least not increased, and the duration of uncomplicated cases is definitely limited.

In summary, then, the uncomplicated cold is a mild disease manifesting itself by a constitutional reaction and by hyperemic phenomena of the upper air passages without local suppurative complications. The duration is brief and there is a tendency to leucopenia.

This primary disease is to be sharply differentiated from certain local complications which occur frequently, and often overshadow the picture, although they are by no means essential but are distinctly secondary. The clinical sequence suggests that the cold itself prepares the ground in some way so that local complications due to pyogenic organisms may arise, and indeed, such complications rarely manifest themselves until the initial disease has been present at least several days. Sinus infections, middle-ear infections, laryngitis, tracheitis and bronchitis, occur frequently—pneumonia and sepsis rarely follow a simple cold. Such processes, once established, may flare up promptly during a subsequent cold and thus serve still further to confuse the picture.

We have then in the case of the cold a perfect analogy to what obtains in epidemic influenza—a primary disease relatively mild in itself, but creating an almost specific predisposition to certain complications in the respiratory tract. The importance of this differentiation between primary disease and complication cannot be overestimated, inasmuch as the bacteriological findings associated with the former and with the latter must be distinguished.

In regard to epidemiology two outstanding facts may be mentioned—the disease is essentially a seasonal one, and one which affects groups of people. At the same time the details of transmission are entirely obscure. Colds are clearly contagious, as evidenced by rapid spread through groups of people, and yet susceptible individuals intimately exposed may escape infection. Similar contradictions have been observed in influenza.

In regard to immunity there is little to be said. Occasional individuals seem to be immune to colds, and they never develop the infection. Others seem highly susceptible and have many attacks during the course of one season. Whether a transient protection is conferred by an attack, we do not know—certainly such protection is usually very temporary.

In summary, then, the common cold falls into a rather sharply defined group of diseases including (1) the common cold, which is endemic and at times mildly epidemic; (2) true influenza which is pandemic; and (3) so-called grippe, intermediate in severity between the cold and influenza, and usually occurring in mildly epidemic form. All these diseases have the following common characteristics:

1. There is a primary disease manifested by a constitutional reaction and by hyperæmic phenomena in the upper air passages.

2. The uncomplicated disease is of self-limited duration.

3. There is usually leucopenia or at least an absence of leucocytosis.

4. Spread of the disease seems to be mainly by direct contact.

5. There is a remarkable tendency to complications in the respiratory tract; in the case of colds to localized infections, such as otitis, sinusitis, laryngitis and bronchitis; in influenza, to broncho-pneumonia as well.

Our purpose in presenting this clinical analysis is to bring out clearly the fact that the common cold is a definite disease of fixed characteristics, to be distinguished sharply from complications which frequently occur during its course. This fact, as well as its striking resemblance to a group of diseases of unknown etiology, should make one cautious in accepting any of the obvious organisms of the throat as its cause.

Literature

The literature on the subject of colds is scant. Even the textbooks have not thought the disease worthy of serious consideration. We find no mention of it, for example, in Reynolds' *System of Medicine,* the standard English work of 50 years ago, or in the more modern system of Osler and McCrae. Mohr and Staehelin are silent on the question, and in Albutt's system a brief and incomplete note is found under the heading of acute rhinitis. In Osler's *Practice* alone have we found anything approaching an adequate discussion of the disease, which the author describes with his usual clearness and accuracy under the heading of acute catarrhal fever.[1] The explanation of this general neglect of so common a malady seems to us to be due again to the lack of general recognition of the common cold

as a definite entity. Almost always the primary disease and its complications are confused. The terminology for example, falls under two heads—first a series of designations which indicate the disease as a whole, and secondly, terms which apply to one phase or complication only. In the former group we find "the cold," "the common cold," "acute catarrh," "acute coryza," and "acute catarrhal fever"; and in the latter "acute rhinitis," "acute pharyngitis," "acute laryngitis," etc. Fantus[2] recently has suggested that colds, grippe and influenza, all be included under the general heading of the catarrhal fevers. There seems to us much reason for such a designation, as it points out the undoubted generic relationship between these various conditions. It would seem wise in the present state of our knowledge to mention both the primary disease and the complication (if present) in writing the diagnosis; for example, "acute catarrhal fever—sinusitis," or "acute catarrhal fever—broncho-pneumonia," whereas in simple cases one would simply say "acute catarrhal fever."

Views as to the Nature of Colds

Aside from bizarre and untenable theories as to the nature of the common cold, usually put forward by a single individual and never gaining general recognition, three main views have prevailed as to the nature of this affection. The first, which was almost universally accepted until the bacteriological era became well established, presumed that the cold was indeed due to exposure to cold or change of temperature in some form. So firmly was this idea fixed in the lay and professional mind that a period of a one hundred years could pass without producing any modification of this doctrine. In 1786, for example, Thomas Hayes in a little treatise entitled "A Serious Address on the Dangerous Consequences of Neglecting Common Coughs and Colds"[3] says "A cold arises from the effect of cold or moist air, applied to the surface of the body and lungs, from going too thinly clad, or exposing the body to cold air, after having been heated by exercise; or when the pores are opened from drinking warm liquors." There follows an admirable clinical description in which the primary disease is well differentiated from its sequels. "A cold, then, is a sense of chilliness on the skin attended with a lassitude and weariness, and slight shivers at times, with a degree of headache, and flying pains in the small of the back and limbs, a stuffing of the nose with or without dry tickling cough or hoarseness. Sometimes the sneezing, stuffing of the nose, or cough, give the first intelligence of its approach, and sometimes it is preceded by some of the other symptoms." One hundred years later Carpentier[4] gives as causes of the common cold—use of tobacco, occupation attended with dust, smoke or irritating gases, excessive dryness or moisture of the atmosphere, sudden changes of temperature, or getting wet feet. "A very pernicious habit is wetting the head, which is practiced daily by young ladies." Exposure to night air, damp clothes, going without a collar, cravat, or cuffs, facing the wind in traveling and not having nose, mouth, throat and chest protected with extra wraps are also listed. Furthermore, certain constitutional causes such as rheumatism and neurasthenia are mentioned. At about the same time Lewisohn[5] claims that colds are due to indigestion, and Ewing[6] that their cause is "interference with nutrition in some part of the body." Hitz[7] finds atmospheric changes a direct factor in the etiology of catarrhal disease of the respiratory tract, and Jack,[8] more recently and to our mind unconvincingly, tries to relate colds to temperature, moisture, and soil conditions.

Although modern writers no longer regard cold as the sole and complete cause of catarrhal affections of the upper air passages, the relationship of exposure and chilling to disease is still the subject of consideration. Schade[9, 10, 11] reports extensive and interesting clinical and experimental observations on the effect of cold on tissues, and its relation to disease. Certain definite effects on the mucous membranes of the respiratory passages seem to be proven. These are reflex vasomotor changes which may lead to anemia or hyperæmia, and also direct irritative phenomena. Schade describes interesting physical changes in tissues which have been affected by cold. In the case of the respiratory mucosa exposure to cold may, according to this observer, produce a so-called "cold catarrh." Its features are onset during or directly after the cold trauma, with rawness and secretion. Objectively, the mucous membrane may appear reddened. There is no fever and the disturbance, which is to be regarded as purely local, lasts only one or two days. Schade believes also that this cold catarrh may allow bacterial invasion with secondary effects. His ideas are supported by an epidemiological study on 17,000 men who were in the trenches on the western front from November 14 to May 17. The first two winters were mild, but during the third, which was severe, the incidence of respiratory infection increased four-fold.

Miller and Noble[12] review the experimental work on the relation of cold to infection and report experiments of their own to the effect that respiratory infection of rabbits with bacillus bovisepticus (snuffles) is favored by chilling, after the animals have been accustomed to heat. Mudd and Grant,[13] and Grant, Mudd and Goldman,[14] showed that chilling of the body surfaces causes vaso-constriction and ischemia in the mucous membranes of the palate, pharynx, and tonsils. In a few cases the flora of the pharynx and tonsils was studied before and after chilling, and apparently there was some increase in certain organisms following the procedure.

On the whole, then, it seems clear that cold may produce an irritation of the mucous membranes of the upper air passages. It also seems quite possible that this injury may be one of a group which alters conditions in such a way as to allow secondary bacterial invasion. How real a part it plays in the production of the true "common cold" cannot be absolutely decided at present, but surely it can only be a contributary factor of the most general sort when one considers the facts of epidemiology and the clinical features of the disease.

With the development of bacteriological knowledge opinion gradually inclined toward infection as playing a part in the production of colds. The effect of cold was still considered important, however, as a predisposing factor following which a bacterial invasion occurred by organisms present in the mouth. James[15] for example, states that colds are caused by

exposure to cold or irritation of the nasal mucous membranes by dust or gas which allows the entry of mouth bacteria. Haig[16] claims that "a cold is excess of uric acid in the blood, plus local action of cold, plus a microbe. The microbe by itself can do nothing"; and Walsh[17] believes that a cold is due to bacterial invasion by organisms present in the mouth following cold or some other injury. This general view seems to have been very popular until recently, and was quite generally accepted. While it marked a distinct advance over the old idea that cold alone was the causal factor, it can hardly, as we shall point out later, explain the facts of epidemic colds.

During the recent years of bacteriological enthusiasm cold has been almost entirely lost sight of in considering the etiology of acute catarrh, and a primary and complete rôle has been ascribed to various bacteria. All of these studies may be criticized for several reasons. In the first place no clinical distinction is made between the primary cold and its complications with consequent confusion of bacteriological results, secondly, no adequate control studies of the flora under normal conditions have been made, and thirdly, the attention of the investigator has very often been confined to only one organism. Cecil[18] for example, in working out the incidence of S. viridans in acute respiratory infections found this organism predominatingly in a large percentage of colds. He fails to recognize it as a normal mouth inhabitant and seems unaware that plates from healthy individuals may often show this organism in almost pure culture. Similarly, Voorhees[19] states that "colds are due to pneumococcus, catarrhalis and other organisms," Mackay[20] finds pneumococcus, B. influenzæ, catarrhalis, staphylococcus, streptococcus and diphtheroids—in other words the entire normal flora of the mouth. Tunnicliff[21] describes a small anaërobic organism, and Floyd,[22] in a study of the streptococcus and pneumococcus groups as found in relation to acute upper respiratory tract infections, encountered these organisms frequently in colds. He makes no attempt to differentiate primary disease and secondary complications, nor does he seem familiar with the normal variations in flora. As far back as 1902 Pfeiffer[23] ascribed colds to the micrococcus catarrhalis which he isolated from catarrhal infections.

In addition to the usual bacteria, filtrable viruses have been discussed as a possible cause of colds. Kruse[24] first brought out this idea. Secretions blown from the nose of a patient with an acute cold were diluted 15 times with salt solution, filtered through a Berkefeld filter and the filtrate was instilled into the nasal passages of 36 healthy people. After an incubation period of two or three days 15, or 42 per cent, of these individuals developed typical colds. Foster,[25, 26] following Kruse and using a similar technique, produced typical colds in at least seven of ten healthy men. After an incubation period of from six or eight to 30 hours dryness of the nose and throat, sneezing, headache, chilliness, flushing, and rhinorrhœa (second day), developed. In six cases there was slight fever—up to 100° F. Other symptoms were sore throat (five cases), pain in the back (four cases), herpes (one case). The duration of the experimental disease was from three to six days. By anaërobic (ascites-tissue) methods Foster cultivated a minute organism, which in turn produced colds on inoculation. Foster's protocols are quite convincing, and one wonders that more effort has not been made by other workers to confirm and extend his work.

Summary

In summary, then, a consideration of the clinical features of colds together with the information available in the literature leads to the following conclusions:

1. The common cold is a definite disease generically related to grippe and influenza. The primary disease is often followed by local complications which tend to overshadow the picture.
2. Cold may produce disturbances in the upper air passages which are to be distinguished from true infectious colds.
3. None of the common bacteria found in the nose or throat have been proven to be the primary cause of colds.
4. The most convincing evidence in the literature favors a filtrable virus as the cause of the common cold.

Present Observations

In a previous paper[27] we reported studies on the normal (aërobic) flora of the throat in healthy individuals. This flora was found to be relatively simple, and practically constant over considerable periods of time. With the background thus obtained it seemed desirable to study respiratory infections to discover what deviation from the norm might be present under conditions of disease. The present report deals with the findings in a series of cases of acute coryza (common cold).

Material

Ten individuals were studied during the months of November and December 1920. At this time colds were very prevalent in Baltimore and the rapid spread of the disease, together with the clinical features, made it clear that we were dealing with the so-called infectious type of acute coryza. Most of our patients had the disease in its uncomplicated form, a few developed secondary local complications. The clinical features will be discussed in detail in connection with the protocols.

Methods

The same methods as those used in studying the normal flora were employed in the present work. Cultures were made at intervals during the active stage of the disease and during convalescence on rabbit-blood agar and oleate hæmoglobin agar. The swabs were taken from the naso-pharynx and tonsils. Cultures were not made from the nose, because we desired to compare the conditions with those previously studied in normals. Furthermore, in addition to draining the nasal mucous membranes, the naso-pharynx was involved in all cases.

The cultures were worked out in considerable detail both quantitatively and qualitatively.

PROTOCOLS

CASE I.—SL

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic Streptococcus	Pneumococcus	Remarks	Clinical notes
Jan. 4...	∞ At least two varieties.	∞	100 cols. (hemolytic).	0	0	Many cols. of a minute Gram-positive organism. A few cols. Staph. albus.	Cold began Jan. 2, nose occluded, throat raw. Tonsils removed. Nasal and pharyngeal mucous membranes reddened.
Jan. 7...	∞	∞	Many cols. (hemolytic).	0	0	....	Convalescent.
Jan. 10..	∞	∞	0	0	0	A few cols. Staph. albus.	Well.

Comment.—Clinically, the case was one of an uncomplicated cold. The members of the normal flora—non-hemolytic streptococcus and Gram-negative cocci were present in large numbers in all the cultures. Staph. albus was present in small numbers in the first and third cultures. In the first culture there were many colonies of a minute Gram-positive organism. Hemolytic influenza bacilli were present in the first two cultures. Our impression of the bacteriology of this case is that we are dealing with the normal flora plus transient insignificant organisms such as one finds in healthy controls. It does not seem possible to assign a causal rôle to any of the bacteria found. The significance of the hemolytic influenza bacillus will be discussed later.

* ∞ = innumerable.

CASE II.—C

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Nov. 12..	∞	∞	100 cols. (hemolytic).	0	0	A few cols. Staph. albus.	Cold began Nov. 12. Stuffiness in nose, sneezing, slight headache, and slight sore throat. No discharge. Exam.: Nose—mucous membrane slightly red and swollen. Throat slightly red.
Nov. 15..	∞	∞	0	0	0	....	Condition about the same.
Nov. 17..	∞	∞	20 cols. (hemolytic).	0	0	....	Feels well.

Comment.—Clinically, the case is one of an uncompleted cold. The bacteriological findings are analogous to those in Case I.

CASE III.—FR.

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Dec. 3...	A few cols.	∞	Many cols. (hemolytic).	0	Many cols.	Many small dewdrop colonies of a Gram-positive coccus. Culture of washed nasal secretion: 6 cols. Staph. albus.	Fourth day of cold. Stuffiness in nose, rawness of throat, marked malaise. Today nose is occluded, the mucous membrane is reddened, pharynx is red. Pale green muco-pus from nose.
Dec. 10..	Many cols.	∞	A few cols. (hemolytic).	0	Many cols.	A few cols. of a Gram-positive diphtheriod.	Practically well. Mucous membranes still a little raw.
Dec. 13..	Many cols.	∞	50 cols. (hemolytic).	0	Many cols.	Many cols. of a hemolytic staphylococcus.	Well.
Dec. 29..	∞	∞	25 cols. (hemolytic).	0	0	....	Well.

Comment.—Clinically, the case is one of an uncomplicated cold. The normal flora—non-hemolytic streptococcus, and Gram-negative cocci—were present throughout, and also a hemolytic influenza bacillus. Pneumococci were present in the first three cultures. A variety of bacteria were temporarily present. The only organism which might possibly be regarded as causal was the pneumococcus present in the first three cultures, but inasmuch as it is frequently found in the mouth and was rarely obtained in the other cases, one can hardly incriminate it as the cause of colds in general. Furthermore, culture from the nasal discharge showed only Staph. albus and no pneumococci.

PROTOCOLS—CONTINUED

CASE IV.—ERB

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Nov. 20..	∞	∞	Many cols. (hemolytic).	0	0	A few cols. Staph. albus.	Onset November 19. Feels badly, throat raw. On exam. pharynx is distinctly reddened.
Nov. 22..	∞	∞	A few cols. (hemolytic).	0	0	....	Full-blown cold. Feels badly, eyes and nose congested. Throat raw, and looks red.
Nov. 23..	Many cols.	∞	Many cols. (hemolytic).	0	0	3 cols. of a yellow hemolytic staphylococcus. ∞ small dew-drop colonies of a Gram-pos. diplococcus.	Condition unchanged.
Nov. 29..	∞	∞	A few cols. (hemolytic).	0	0	Many small dew-drop colonies of a Gram-pos. diplococcus. 20 colonies of a "spreader," many colonies of a yeast.	Condition much better. Throat still feels raw. Appearance of throat about normal.
Dec. 2...	Many cols.	A few cols.	200 cols. (hemolytic).	0	0	100 cols. of a Gram-pos. diphtheroid. ∞ small dew-drop colonies of a Gram-pos. diplococcus. ∞ Cols. of yeast.	Exacerbation yesterday, feels badly, nose completely occluded, eyes running, moderate amount of mucoid discharge from nose.
Dec. 4...	∞	A few cols.	20 cols. (hemolytic).	0	0	A few small dew-drop colonies of a Gram-pos. diplococcus Many cols. of a Gram-pos. diphtheroid. ∞ cols. of a yeast.	Condition unchanged.
Dec. 10..	∞	∞	50 cols. (hemolytic).	0	0	A few small dew-drop colonies of a Gram-pos. diplococcus A few small tough colonies. of a minute Gram-positive organism.	Practically well. Mucous membranes still raw.
Jan. 3...	Many cols.	∞	100 cols. (hemolytic).	0	0	A few small tough colonies of a minute Gram-pos. organism.	Well.

Comment.—Clinically, the case is one of a severe but uncomplicated cold. The bacteriological findings are of special interest. The normal flora as well as a hemolytic influenza bacillus were present in all the cultures. Staph. albus, Staph. aureus, and a spreader were present as transients in a single culture. A Gram-positive diphtheroid was present on two successive cultures but not before or afterwards. A minute Gram-positive organism was present in the last two cultures. In four successive cultures from November 29 to December 10 a Gram-positive diplococcus, growing in minute dew-drop colonies, was obtained, and in three successive cultures a yeast grew in very great numbers. It does not seem possible that any of these organisms was the cause of the cold. The yeast " infestation " seems to us of special interest. This organism practically never occurs in the mouth, and, as far as we know, never produces disease there, yet E. carried it in tremendous numbers for over a week. Some sort of a biological adaptation must have taken place, for experimental observations show that organisms otherwise disappear much more rapidly. The possibility that the alteration of the mucous membrane during the cold allows a temporary adaptation of various bacteria must be considered.

E.'s flora was studied from March 4 to April 25, 1920. It is interesting to compare the findings at that time with those present during his cold six months later. In the former series hemolytic influenza bacilli were present in two of ten cultures, and non-hemolytic influenza bacilli in four. During the recent series no non-hemolytic influenza bacilli were found. The transient flora last spring was less varied than during this winter. For details, see Case IX in the previous report (ref. 27).

CASE V.—M

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Dec. 9...	A few cols.	∞	40 cols. (hemolytic).	0	A few cols.	....	Fifth day of cold. Moderate general symptoms. No complications. Slight mucoid nasal discharge. Throat slightly red.
Dec. 11..	A few cols.	∞	50 cols. (hemolytic).	0	0	....	Cold worse, but no complications.
Dec. 14..	Many cols. (three kinds).	∞	6 cols. (hemolytic).	0	0	....	Well.
Jan. 8...	∞	∞	0	0	0	....	Well.

Comment.—Clinically, the case is one of an uncomplicated cold. The normal flora was present throughout. Hemolytic influenza bacilli were obtained in three of four cultures. A few colonies of pneumococcus were obtained in the first culture but not thereafter.

The flora of this individual was studied about six months ago. At that time no hemolytic influenza bacilli were obtained, but non-hemolytic influenza bacilli were present in four of six cultures.

PROTOCOLS—Continued

CASE VI.—L

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Dec. 1...	∞	∞	50 cols. (hemolytic).	0	0	∞ dew-drop cols. of a gram-pos. coccus. Many huge flat colonies of a gram-pos. streptococcus.	Second day of cold. Stuffiness in nose; throat sore. Slight malaise.
Dec. 9...	∞	∞	10 cols. (hemolytic).	0	0	A few huge flat colonies of a gram-pos. streptococcus. Culture from tracheal mucus; mixed flora—same as mouth.	Feels better. Tracheitis with sputum for several days.
Jan. 19..	∞	Many cols.	∞ (hemolytic).	0	0	....	Well.

Comment.—Clinically, the case is one of a cold complicated by a tracheitis. The normal flora and also hemolytic influenza bacilli were constantly present. On the first culture innumerable colonies of a Gram-positive coccus were obtained. On the first two cultures an unusual non-hemolytic streptococcus growing in huge (3–5 mm.) flat colonies was obtained. Culture from the tracheal sputum showed no specific organism.

CASE VII.—T

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Nov. 12..	A few.	200 cols.	20 cols. (hemolytic). ∞ cols. (non-hemolytic).	0	0	Many colonies of a coarse Gram-pos. coccus. ∞ cols. of a minute Gram-pos. organism (pleomorphic bacillus?). 20 cols. Staph. albus. A few cols. of a Gram-pos. bacillus in chains.	Onset Nov. 10, began with irritation in nose and slight sore throat. Today feels very badly, fever and aching. Clear discharge from nose. Throat a little red. Tonsils removed.
Nov. 15..	∞	∞	3 cols. (hemolytic). Many cols. (non-hemolytic).	0	0	Many colonies of a coarse Gram-pos. coccus. A few cols. Staph. albus.	Cold still in active stage. Nose running, throat raw. Cough with muco-purulent expectoration.
Nov. 17..	A few.	∞	Many cols. (hemolytic).	0	0	∞ colonies of a Gram-pos. diplococcus. ∞ cols. of a minute Gram-pos. organism.	Better. Throat still raw. Moderate muco-purulent expectora-
Nov. 19..	Many cols.	∞	A few cols. (hemolytic). Many cols. (non-hemolytic).	0	0	....	Improving, throat still raw. Less cough and sputum.
Nov. 30..	Many cols.	∞	50 cols. (hemolytic). Many cols. (non-hemolytic).	0	0	Many cols. of a Gram-pos. diplococcus. 10 cols. Staph. albus.	Well.
Dec. 13..	A few.	∞	6 cols. (hemolytic).	0	0	Many cols. of a Gram-pos. diphtheroid.	Well.
Jan. 19..	∞	∞	100 cols. (hemolytic).	0	0	A few cols. of a Gram-pos. diphtheroid.	Slight sore throat.

Comment.—Clinically, the case is one of a cold complicated by tracheitis. The normal flora as well as hemolytic influenza bacilli were constantly present. Non-hemolytic influenza bacilli were obtained in four of seven cultures. Various organisms were present as transients.

CASE VIII.—BL

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Dec. 21..	0	∞	0	0	0	Several hundred cols. of a coarse gram-pos. coccus; 15 cols. of a yellow staph.	Third day of cold. Nose stuffy, throat raw. Malaise and chilliness. For one day tracheitis with thick green purulent sputum.
Dec. 29..	∞	∞	0	0	0	A few cols. of a coarse gram-pos. coccus; 15 cols. of a yellow staph.	Practically well. Throat still a little raw, slight cough with mucoid sputum.
Jan. 8...	Several kinds.	∞	0	0	0	Many cols. of a yellow staph.	Well. Still very slight throat catarrh.
Sputum culture Dec. 21.	0	0	0	0	∞	Many cols. of a coarse gram-pos. coccus.	

Comment.—Clinically, the case is one of a cold complicated by a severe tracheitis. The normal flora was constantly present. This is the only case in the series in which hemolytic influenza bacilli were absent. Two organisms which are not normally present in the throat were obtained—a yellow staphylococcus, and a coarse Gram-positive coccus. From the sputum a pneumococcus was obtained in almost pure culture. This organism was clearly associated with the tracheitis.

PROTOCOLS—Continued

CASE IX.—O

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Nov. 5...	A few cols.	∞	100 cols. (hemolytic).	0	0	....	Onset Nov. 3. Fullness in nose and sneezing. To-day (3d day) head feels congested and nose is stopped up. Slight mucoid discharge. Throat not sore. Does not feel ill. Exam.—nasal m. m. red and swollen, large tonsils, pharynx injected.
Nov. 6...	A few cols.	∞	Many cols. (hemolytic).	0	0	....	Condition unchanged.
Nov. 8...	A few cols.	∞	Many cols. (hemolytic).	0	0	....	Condition unchanged.
Nov. 12..	∞	∞	20 cols. (hemolytic).	0	0	....	Nose clear. Says left tonsil is sore, but it does not look remarkable.
Nov. 15..	0	∞	∞ cols. (hemolytic).	cols. β type.	0	....	Says throat has felt very raw for three days. Looks a little red.
Nov. 16..	A few cols.	∞	∞ cols. (hemolytic).	∞ cols. β type.	0	....	Throat quite raw and sore, looks a little red, no exudate. No fever, but feels chilly.
Nov. 19..	∞	∞	3 cols. (hemolytic).	0	0	20 large grey-green slightly hemolytic cols. of a long-chained streptococcus. Many minute tough grey colonies of a small Gram-pos. organism.	Throat still a little sore, but the patient is practically well. Throat looks normal.
Dec. 14..	Many cols.	∞	∞ cols. (hemolytic).	0	0	Many colonies of a Gram-pos. diphtheroid.	Cold well. Right side of throat has been sore from time to time.

Comment.—Clinically, the case is one of a cold complicated by tonsillitis. The normal flora and also hemolytic influenza bacilli were present throughout. On the tenth day, the patient developed a sore throat and coincidently innumerable colonies of a hemolytic streptococcus were obtained, which disappeared as the tonsillitis subsided.

CASE X.—R

Culture	Non-hemolytic streptococcus	Gram-neg. cocci	B. influenzæ	Hemolytic streptococcus	Pneumococcus	Remarks	Clinical notes
Dec. 8...	Many cols.	∞	0	0	Many cols. Type IV.	∞ cols. Staph. albus. Many cols. of a minute Gram-pos. organism. Sputum culture: Pneumococcus, Type IV (pure culture).	Cold began one week ago. Rhinorrhoea, slight sore throat, moderate general symptoms. On 4th day developed cough and sputum. Feels better now, but severe cough with thick green purulent sputum persists. On examination throat is slightly red.
Dec. 11..	Many cols.	∞	0	0	A few cols. Type IV.	∞ cols. Staph albus. Sputum culture: Pneumococcus, Type IV (pure culture).	Condition unchanged.
Dec. 14..	∞	∞	0	0	0	∞ cols. Staph albus. Sputum culture: Many cols. Staph albus. A few cols. M. catarrhalis. Many cols. of non-hemolytic strept.	Feels better. Cough much diminished. Only small amount mucoid sputum now.
Dec. 17..	Many cols.	∞	Several hundred cols. (hemolytic).	0	0	∞ cols. Staph albus. A few cols. of a minute Gram-pos. organism. Many dew-drop brownish cols. of a Gram-pos. coccus.	Feels well. No sputum. Persistent nasopharyngeal drip.
Dec. 20..	Many cols.	∞	Many cols. (hemolytic).	0	0	∞ cols. Staph albus. Many dew-drop brownish cols. of a Gram-pos. coccus.	Condition unchanged.
Jan. 4...	A few cols.	Many cols.	Many cols. (hemolytic).	0	0	∞ cols. Staph albus.	Condition unchanged.

Comment.—Clinically, the case is one of a cold, complicated by bronchitis. The normal flora was constantly present. From the bronchial pus a pneumococcus, Type IV, was isolated in pure culture. This was clearly associated with the complicating bronchitis. On the fourth culture hemolytic influenza bacilli were obtained and thereafter they were constantly present.

Innumerable colonies of a white staphylococcus were present throughout. As this is a distinctly abnormal finding, another complication was suspected. It was found that the patient had a chronic ethmoid infection for which surgical treatment had been advised by Dr. Crowe.

Results

With the knowledge previously gained of the normal throat flora as a background, the present cases were studied to determine any variations from the normal which might occur during the course of a cold, and to see if such variations might be interpreted in such a way as to shed light on the etiology of the disease.

In the uncomplicated cases the outstanding fact was this, that the flora differed in no fundamental way from that which obtains in healthy individuals. As in normal controls, non-hemolytic streptococci and Gram-negative cocci are constantly present. As in the normal, there is a shifting transient flora consisting of various organisms—diphtheroids, coarse Gram-positive cocci, minute Gram-positive organisms, white staphylococci and others. Two points in regard to this transient flora are, however, worthy of note. It is distinctly richer and more varied than that found in our series of controls, and in some cases organisms persisted for considerable periods of time—as in the case of the yeast infestation in E. A conceivable explanation of these findings is that the disturbance of the mucous membranes during the cold allows a general increase in the activity of bacterial growth on these surfaces. This fits in with the old theory that the cold, whether produced primarily by cold or by infection, leads to environmental changes which, as it were, light up the bacterial flora already present in the mouth. However, we feel definitely that none of these organisms can be the primary cause of the cold because their presence is too variable and inconstant, and the variety of them is great.

The almost constant presence of hemolytic influenza bacilli was of interest. These organisms were absent in only one case of the series. At first one was tempted to relate them to the cold in an etiological way, but it soon appeared that they could be nothing more than saprophytes, inasmuch as they were present in equal numbers in unaffected controls. Their frequency, however, illustrates one of the grave pitfalls of respiratory bacteriology.

The cases showing a clinical complication were of great interest, for here, in practically every instance, it was possible definitely to find that an organism which is not normally present was producing the complication. In O. a tonsillitis was associated with a hemolytic streptococcus, in B. and in R. bronchitis was associated with a pneumococcus, and in R. a sinus infection with Staph. albus.

Discussion

On clinical grounds the view was advanced that the common cold is an infectious disease analogous to influenza, featured by the frequent development of complications in the upper air passages such as sinus infections, tracheitis, and otitis. A review of the literature showed no convincing evidence that any known organism is the primary cause of the cold.

The cultural studies in the present report fail to show in uncomplicated cases any variation in the flora which would enable one to select any organism or group of organisms as the cause of colds. On the other hand, where clinical complications occurred pathogenic organisms were definitely associated with them.

We feel therefore that the primary cause of colds is probably an organism as yet unknown and certainly not one of the usual pathogens such as Streptococcus, Pneumococcus, B. influenzæ, or Staphylococcus. But the primary cold, whatever its final cause, alters the mucous membranes in such a way as to allow secondary bacterial invasion and consequent frequent development of local complications. The cultures clearly indicate that such complications are due to a variety of bacteria such as Pneumococcus, Streptococcus, and Staphylococcus.

In general it seems that the method of serial comparative study is necessary in working out the bacteriology of respiratory infections. Such a method allows one to pick out and interpret the significance of unusual organisms and also checks premature conclusions as to the etiological bearing of such organisms.

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HEMOLYTIC INFLUENZA BACILLI

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Members of the pneumonia commission working at Fort Sam Houston in 1918 noticed, in throat cultures from patients with measles, colonies of Gram-negative bacilli surrounded by a zone of hemolyzed red blood cells. The incidence of hemolytic streptococci in throats was being studied at the time and the similarity in the types of hemolysis caused by these two organisms was striking enough to be of interest. Later some of these men, studying measles, influenza and pneumonia at Camp Pike, encountered this same Gram-negative hemolytic bacillus in throat cultures. They recognized that the types of hemolysis caused by this bacillus and by the hemolytic streptococcus were similar, but that the growths were different, the former making clear dew-drop colonies, the latter opaque, whitish colonies. They also noticed that the bacillus would not grow in meat-infusion broth.

Very little can be found in the literature about Gram-negative, non-motile, aërobic, hemolytic, hemoglobinophilic bacilli. Pritchett and Stillman[1] recovered an organism of this kind from throat cultures and called it "Bacillus X." Evidently at that time they did not consider it in the B. influenzæ group, as they say, "The colonies most readily confused on inspection with those of B. influenzæ were those of Micrococcus catarrhalis, Meningococcus, diphtheroids, and especially an unidentified organism called 'Bacillus X.'" In a previous paper on the biological classification of influenza bacilli, one of the authors,[2] after showing that B. influenzæ could be differentiated from B. pertussis culturally and that the former group could be sub-divided culturally, reported having studied some of the hemolytic hemoglobinophilic bacilli and having found that they, also, were not all alike culturally, and at that time suggested that they might be classified as hemolytic influenza bacilli. In a recent paper by Stillman and Bourn[3] all of the above statements have been confirmed and much valuable information has been added. Nevertheless they speak of non-hemolytic hemoglobinophilic bacilli as B. influenzæ and hemolytic hemoglobinophilic bacilli as "Bacillus X."

Pfeiffer[4] originally described a small, Gram-negative, non-motile, aërobic, hemoglobinophilic bacillus which he called the influenza bacillus. Then he found the pseudo-influenza bacillus. The distinction was made on morphology alone. Later he decided there was no real difference between the false and the true forms and that all influenza bacilli were alike. This view cannot be held at present for there is enough evidence to show that these bacilli are not all alike even in their cultural characteristics. In fact, all Pfeiffer did was to discover a new group of organisms and call them influenza bacilli. The question of hemolysis did not enter into the earliest descriptions of these bacilli. That point would have been hard to determine as they were first cultured by streaking bloody or purulent sputum on hardened agar plates and later by streaking a pure culture with blood on the surface of agar. From later descriptions it can be gathered that they were non-hemolytic, at least all bacteriologists considered them so. There is, however, a group of bacilli which coincides in every respect with the meager description given to influenza bacilli by Pfeiffer with the exception that they are hemolytic. The object of this paper is to show, if possible, that they belong in the large group of influenza bacilli just as hemolytic and non-hemolytic streptococci fall into the great group of streptococci and to suggest that bacteriologists in the future speak of hemolytic and non-hemolytic influenza bacilli.

In October, 1919, the throats of forty normal medical students were swabbed and from twelve (30 per cent) a Gram-negative, hemolytic, hemoglobinophilic bacillus was recovered. Later these cultures were lost, but during the epidemic of influenza in the early part of 1920, from 25 per cent of 52 throat cultures, 13 strains were isolated from influenza patients. None was isolated from the lungs or from blood cultures at autopsies, and so far as is known, they are not pathogenic for man. These strains have been studied but all have not been carried through, as some have died. However, five strains have been successfully carried through all the work and have been under constant observation for nine months.

All the throat cultures were made on 2 per cent fresh rabbit blood meat-infusion agar, pH 7.5. Both the hemolytic and the non-hemolytic groups grow well on this medium and they can be differentiated immediately, whereas it is impossible to do so on the oleate or cooked blood media. In a previous paper Rivers[5] showed that fresh human blood inhibited markedly the growth of B. influenzæ. Those were non-hemolytic. The same, however, is true of the hemolytic group and their growth is inhibited to such an extent by fresh human blood that it is impossible to use it in the work of isolation of these organisms with any degree of satisfaction. Fresh rabbit or cat blood, 2 per cent, can be used and as far as growth is concerned is just as satisfactory as the oleate or cooked blood media; moreover in other respects it is more satisfactory, since the hemolytic and non-hemolytic bacilli can be differentiated on the

original plates, giving the relative number of the two organisms present.

Naturally, the first question to be answered is, Are these bacilli hemoglobinophilic? Much work has been done to show that this is untrue of Pfeiffer's influenza bacillus and it is to be supposed that the same objections will be raised in regard to these hemolytic bacilli. Anyway, it can be said that one group is as hemoglobinophilic as the other. Pritchett and Stillman,[1] Stillman and Bourn[3] and one of us (Rivers)[2] have found them so. Meat-infusion agar or broth, pH 7.1-7.5, enriched with blood free ascitic fluid, is lacking in something necessary for growth, yet growth takes place in almost any kind of medium if a very little blood is added. The amount of blood necessary is very small and at times is hard to detect after it has been added. The hemolytic as well as the non-hemolytic bacilli show the phenomenon of symbiosis on unfavorable media and like the non-hemolytic ones will also grow for some generations on hemoglobin-free media in symbiosis with the proper organisms.

When 2 per cent rabbit blood meat-infusion agar plates were inoculated with these 13 strains, within 24 hours a zone of hemolyzed cells could be seen immediately around the streaks or the individual colonies. The cells were actually destroyed and the hemoglobin had diffused through the medium causing a deeper red color at the junction of the hemolyzed with the unhemolyzed portions of the plates. It was the beta type of hemolysis as applied to hemolytic streptococci. Some strains constantly formed wider zones of hemolysis than others but the type was always the same, the difference being merely one of degree. Hemolysis occurred in liquid as well as on solid media. After the cultures in liquid media were about a week old, long after the hemolysis had been completed, some would develop a greenish brown, others a dirty blackish brown color, and in some there was a fine whitish sediment sticking to the sides of the tubes, seen best at the top of the cultures.

Apparently a free hemolysin was demonstrated by growing the bacilli in "chocolate blood" meat-infusion broth. When the blood was coagulated and precipitated by heat a fairly clear medium was produced which could be used for the study of free hemolysin formation. Eight- to twelve-hour cultures were centrifuged till the supernatant fluid was clear. Half of the supernatant fluid was heated at 56° C. for 30 minutes and then to both portions 1 per cent washed rabbit cells were added and incubated at 37° C. for several hours. Slight hemolysis occurred in the unheated portion whereas none appeared in the heated one. Filtering the cultures through Mandler filters removed much of the hemolysin, but its presence was thought to have been demonstrated several times.

The addition of dextrose to the medium prevents hemolysis of red blood cells by hemolytic streptococci. Four strains of these hemolytic bacilli were grown on solid and in liquid media with and without dextrose to determine whether hemolysis could be influenced. The hemolysis produced by three of them was retarded and diminished in amount by the presence of dextrose; that produced by the fourth strain did not seem to be affected. All four strains fermented dextrose, but the fourth did so slowly, and hemolysis was not affected, while the others fermented the sugar fast enough to diminish the hemolysis but not fast enough to inhibit it completely as is the case with hemolytic streptococci. Dextrose should be omitted from all media which are used to show the presence or absence of hemolysis by members of the hemoglobinophilic group.

Pfeiffer's bacillus has been spoken of by many as a strict aërobe. In the seventh edition of the text-book by Park and Williams[6] it is called a facultative anaërobe. It seemed interesting to determine what relationship there is between the hemolytic and the non-hemolytic groups in regard to this matter. Meat-infusion agar with 2 per cent rabbit blood was inoculated with five of the hemolytic strains and one meningitic strain obtained from Dr. Wollstein. The cultures were put in a large vacuum jar, the air was exhausted (29-30 inches) three times, the jar being filled, between the evacuations, with hydrogen from a Kipp generator. After 24 hours' incubation the vacuum was tested and found to be satisfactory. The cultures were examined and a good growth was obtained in all. It seems from this that the hemolytic as well as the non-hemolytic bacilli are facultative anaërobes.

The colony formation of both groups, hemolytic and non-hemolytic, is almost identical, and if it were not for the hemolysis, it would be impossible in many instances to distinguish one from the other. Some of the hemolytic ones form large colonies which are more or less uniform in size; others grow in small uniform colonies, while still others show an irregularity in colony formation. Young colonies are clear and oval with smooth edges. Older colonies have brownish granular centers, and become checkerman, cone, or truncated cone-shaped, with irregular edges due to the formation of daughter colonies. Some are moist and soft, others somewhat tough, but all of them leave a shallow pit in solid media when a colony is moved. Streak cultures have lobate edges.

It is rather difficult to discuss the morphology of either the hemolytic or the non-hemolytic group, as this is rarely the same twice in succession. In general it may be said that the hemolytic bacilli are slightly longer, heavier, show squarer ends, and stain more deeply and regularly than the non-hemolytic ones. Some, however, are small cocco-bacilli and as far as morphology is concerned cannot be distinguished from those of the non-hemolytic group. No motility has been observed in 8- to 10-hour blood-broth cultures. All take the ordinary stains fairly well but irregularly, are distinctly Gram-negative, and do not form spores.

Blood-broth tubes inoculated with the 13 strains of hemolytic bacilli were incubated for varying periods of time, three to ten days. Then they were extracted with a small amount of ether. This ether extract was decanted and layered with Ehrlich's reagent. Three strains always gave a positive test for indol, the other ten were constantly negative.

Tubes of potassium nitrate blood-broth inoculated with the 13 strains were incubated for five days and then tested with the sulphanilic acid and naphthylamin reagent for nitrites. The uninoculated control tube gave a negative result, but all

of the inoculated tubes gave a positive test, a deep pink or reddish color showing the presence of nitrites.

Five of the hemolytic strains were grown in milk to which a little blood had been added. All produced alkali. Pritchett and Stillman [1] showed this previously. Many of the non-hemolytic ones, if not all, also produce alkali in blood-milk mixtures.

In another paper Rivers,[2] early in the work, stated that none of the hemoglobinophilic bacilli fermented certain sugars. This is incorrect, although he was unable at that time to demonstrate it. This point will not be taken up here but will be dealt with in a later paper. This much can be said; both the hemolytic and the non-hemolytic groups ferment certain sugars. This has been well shown by Stillman and Bourn.[3] There is nothing in the sugar fermentations to prevent the hemolytic group from being called influenza bacilli.

DISCUSSION

Thus far no reason has been found why the hemolytic group of these hemoglobinophilic bacilli should not be called influenza bacilli just as much as the non-hemolytic ones. There is no proof that some of the original organisms described by Pfeiffer as B. influenzæ did not belong to the hemolytic group. Very likely, using the methods described early by him, he worked with both groups and thought they were alike.

An interesting observation was made during the course of the work. One of the 13 strains, after cultivation for seven months on artificial media, lost completely its ability to hemolyze blood both on solid and in liquid media, while retaining all of its other cultural characteristics unchanged. Enough was known of its sugar fermentations and other cultural characteristics to exclude the possibility of a mistake. Any doubt is further excluded by the fact that the hemolytic stock strains were kept separate and transferred at a different time from the non-hemolytic ones. If the classification suggested, or implied, by Stillman and Bourn [3] be followed, the designations to be given to this strain would also imply fundamental differences in its character which its history denies. The organism, once a hemolytic hemoglobinophile, would be called "Bacillus X" of Pritchett and Stillman,[1] now, a non-hemolytic hemoglobinophile, should be called B. influenzæ. This is not believed to be the case. It has always been an influenza bacillus. Once it had many cultural characteristics, one of which was the ability to hemolyze red blood cells. Now it is the same bacillus except it has lost one of its many cultural characteristics, its power to produce hemolysis.

CONCLUSION

On account of the meager description given to the original B. influenzæ, especially concerning hemolysis, it seems best to regard both hemolytic and non-hemolytic aërobic, non-motile, Gram-negative, hemoglobinophilic bacilli as influenza bacilli and disregard any such confusing terms as "Bacillus X" which in itself represents not one organism but a group. After all, it makes very little difference what any of these bacilli, non-hemolytic or hemolytic, are called, so long as every-one recognizes that they belong to the same big group.

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EXPERIMENTAL OBSERVATIONS ON BONE MARROW

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The deposition of pigments in the bone marrow under physiological and experimental conditions has been studied by a number of investigators. Ponfick (1869) and Hoffmann and Langerhans (1869) observed the intracellular deposition of granules of cinnabar in the bone marrow after injecting it into the circulation of the living animal. They thought that the cells concerned in the phagocytosis of particulate matter in the bone marrow were lymphocytes. Cousin (1898) injected suspensions of carmine or litmus blue into animals and made the important observation that the pigment was phagocytosed by the endothelial cells lining the capillaries of the bone marrow. Ribbert (1904), using lithium carmine, confirmed Cousin's observations. Nasse (1876) and Quincke (1880) described the normal presence of pigment in the bone marrow of different animals, but it remained for Brass (1913) to demonstrate that this material is also stored within endothelial cells.

Finally, Evans (1915), using vital dyes such as trypan blue and pyrrhol blue, found that these also were deposited in the bone marrow. He observed, however, that although a large quantity of the dye was concentrated in the cytoplasm of endothelial cells, there were other phagocytic cells in the marrow—the so-called reticulum cells—whose connection with the endothelium is not clear. Recent experiments by Nagao (1920), who injected a suspension of carbon particles into the blood-stream of rabbits and guinea-pigs, confirm Evans' observation that, in the marrow, reticulum as well as endothelial cells participate in the phagocytosis of foreign particles.

The observation that the endothelial and reticular cells of the bone marrow phagocytose solid particles brought to them by the circulating blood led to the present experiment. It

was known that is was possible to inject into the blood stream of the living animal a pigment which would be phagocytosed by the marrow cells, and that after the animal was killed the bones of the entire body could be decalcified and the tissues cleared by the Spalteholz method. It was hoped that in this way the bone marrow of the entire body would be delineated by the presence of the opaque pigment in its cells.

The first series of experiments was performed on rabbits. The pigment used was a suspension of carbon particles, available in ordinary India ink. The ink, diluted one-half with distilled water, was injected into the ear vein of adult rabbits in doses of from 5 to 10 c. c. in two successive days. New-born rabbits received 0.5 c. c. on two successive days. Two or three days after the last injection the animal was killed.

At autopsy the characteristic distribution of carbon particles in the body was observed. The carbon was confined practically to the liver, spleen, lungs, and bone marrow, to which tissues it imparted a gray or black appearance readily visible to the naked eye. On gross inspection the bone marrow appeared conspicuously black. The remaining tissues of the body were normal in color. Microscopic examination showed that carbon granules were abundantly present in the liver, spleen, lungs, and bone marrow. In the liver they were deposited in the Kupffer cells; in the spleen, in the reticulo-endothelial cells; in the lungs, in macrophages, possibly of endothelial origin; in the bone marrow granules were observed, for the most part intracellular, in the endothelium of the vessels and in the cells of the reticulum, although here and there granules were encountered lying free in the vascular channels.

It is interesting to note that the carbon particles, administered in the quantities named, produce no serious effects on the animal's health. The animals may be allowed to live for periods of months without showing any symptoms attributable to the foreign particles stored in the liver and other organs. Gross and microscopic examination of the tissues after a period of months reveals that the carbon deposited in endothelial cells has not diminished in amount nor produced any pronounced pathological changes. It appears that the inert foreign material is permanently stored in the endothelial cells without severe damage to the tissue, much as carbon is deposited in the lungs by inhalation.

The animals which were injected for clearing were sacrificed on the third or fourth day following injection. The bodies were skinned, eviscerated, and fixed in 10 per cent formalin. After fixation the bones were decalcified by placing the bodies in 5 per cent nitric acid until decalcification was complete. The specimens were then washed thoroughly, bleached with hydrogen peroxide, and dehydrated by alcohol. They were finally cleared by the method described by Spalteholz (1914), which consists in transferring them from absolute alcohol to benzol for 48 hours, then into several changes of oil of wintergreen and one part of benzyl benzoate, in which they are kept permanently. In the specimens prepared in this way the bones and tissues surrounding them become perfectly transparent, while the bone marrow stands out conspicuously as deep black patterns (Figs. 1 to 5).

The findings in the adult skeleton will be first described. Carbon was abundantly present in the cervical, thoracic, lumbar, sacral, and caudal vertebræ. It extended from the bodies of the vertebræ through the pedicles into the vertebral arches, whence it was continuous with deposits of carbon in the transverse and spinous processes. The carbon consequently completely outlined the shape of the vertebræ.

Carbon was deposited heavily and uniformly throughout the ribs. On close inspection the rib marrow appeared fenestrated. The sternebræ were also conspicuously black. The sixth segment of the sternum, the xiphoid process, exhibited an elongated black strip of marrow, to which a broad, flat plate of colorless cartilage was attached.

In the skull the deposition of carbon occurred in both membrane and cartilage bones. It was confined, however, to those bones which are in whole or in part cancellous. Thus, the occipital, posterior sphenoidal, anterior sphenoidal, interparietal, parietal, and frontal bones stood out prominently as a result of the black pigmentation between the outer and inner tables (Figs. 4, 6, 7, 8). In other bones, namely the squamosal, the petrotympanic, the maxillary, the zygomatic, and the mandible, in which the cancellous portions are restricted, pigmentation was scant. In the ethmoidal, inferior turbinate, premaxillary, nasal, lacrimal, palatine, and vomer bones no deposition of carbon granules was observed. The distribution of carbon granules in the bones of the skull is diagrammatically shown in Figures 6, 7, and 8.

The hyoid bone was deeply pigmented, as were also the lesser and greater cornua, two independent elements which articulate with it.

The deposition of carbon in the scapula was confined, as in the bones of the skull, to the cancellous regions. Thus, the head and neck of the bone, the axillary and superior borders, the scapular spine, the acromion and metacromion, were deeply pigmented. The vertebral border and the supraspinous and infraspinous fossæ were transparent. The clavicle, which in the rabbit, as in many other mammals, is an imperfectly developed bone, was visible as a narrow black line attached to the sternum by the sterno-clavicular ligament.

The humerus, radius, and ulna were completely outlined by a continuous, opaque black mass. The carpal and metacarpal bones and the phalanges of the digits contained only traces of carbon.

The coxal bone was clearly outlined as a heavy black structure. The femur, tibia, and fibula were deep black. The tarsal and metatarsal bones and phalanges contained traces of carbon.

In a three weeks' old rabbit the distribution of carbon differed somewhat from that in the adult (Fig. 5). The heaviest pigmentation occurred in the long bones of the extremities. The carpal, metacarpal, tarsal, and metatarsal bones, and the phalanges were deeply pigmented. In the long bones, in which the epiphyses and diaphyses were still ununited, carbon was visible in the cancellous portions of the epiphyses as well as in the shafts of the bones. The coxal bone contained three distinct masses of carbon corresponding to the ilium, ischium, and pubis. The distribution of carbon in the scapula

resembled that in the adult. The clavicles were visible as two thread-like lines. The hyoid bone contained a tiny black center. In the skull only traces of carbon were visible in the as yet only partially ossified occipital, parietal, frontal, superior maxillary, zygomatic, temporal, posterior sphenoidal, anterior sphenoidal, and mandibular bones.

In a rabbit one week after birth carbon particles were abundantly phagocytosed and stored in those bones in which ossification had commenced. Thus the separate ossification centers of the vertebræ stood out as well-defined black masses. The ribs each contained a prominent band of black marrow, and there was abundant carbon in the sternebræ. The bones of the extremities, in which ossification had commenced, were filled with carbon-colored marrow. In the skull the deposition was limited to portions of the basioccipital, the exoccipital, the supraoccipital, the basisphenoidal, the presphenoidal, the squamosal, and the mandibular bones.

The different bones were examined microscopically.. Clusters of blood-forming cells occurred fairly uniformly throughout the cancellous portions and shafts of all the bones and consequently in the rabbit a distinction between yellow and red marrow could not be made. Particles of carbon were abundantly present in the cytoplasm of reticulum cells and in the endothelial cells lining the marrow capillaries. The distribution of carbon particles was found to coincide closely with that of the hemopoietic tissue. In the adult animals carbon was found nowhere else than in the marrow of the bones, but in the developing animals a few granules of carbon were observed in some of the osteoblasts in the regions of the epiphyseal lines.

From these observations it will be seen that the bone marrow of the rabbit contains cells which are extremely phagocytic toward inert particulate material afloat in the blood stream. Some of these are endothelial cells which line the vascular channels of the marrow, others are reticulum cells which form the supporting tissue for the blood-forming elements of the marrow. This phagocytic power is present at birth. Microscopic examination reveals that the distribution of these phagocytic cells in the bones closely coincides with that of the blood-forming elements. The cleared tissues of rabbits injected during life with carbon particles give us an accurate picture of the gross distribution of the marrow. The amount of marrow in flat bones is in direct proportion to the amount of cancellous or spongy structure which the bone possesses.

As the experiments described above were confined to the rabbit, it was deemed important to observe the behavior of the bone marrow of other mammalia towards particulate matter, for the purposes of comparison. To this end several dogs, cats, and guinea-pigs were similarly injected with suspensions of carbon. In the dog and cat carbon was not grossly visible in the marrow, and on microscopic examination only an occasional carbon particle could be discovered in the endothelial cells. In the guinea-pig the marrow appeared in the gross definitely blackened by the presence of the carbon, but not to the same extent as in the rabbits receiving corresponding injections. Microscopically the marrow of the guinea-pig was found to contain numerous carbon-laden phagocytic cells, less numerous, however, than in the rabbit.

There exists, therefore, a difference in the ability of the bone-marrow cells in different mammalia to phagocytose and store particles of carbon. In the cat and dog the storage of inert particulate matter, so far as can be determined by quantitative observation, is confined to the liver, spleen, and lungs; in the guinea-pig the bone marrow plays an additional though subordinate rôle; while in the rabbit the material is distributed equally between the liver, spleen, lungs, and bone marrow.

The method herein described of observing the distribution of inert particles in cleared bones may prove valuable in the study of the pathology of bones. It would be of interest to use this method in the experimental study of healing fractures and in experimentally produced disturbances of bone during development and growth.

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DESCRIPTION OF PLATE

FIG. 1.—Lateral view of thorax of a rabbit decalcified and cleared by Spalteholz's method, showing distribution of carbon in the ribs.

FIG. 2.—Anterior view of thorax of a rabbit decalcified and cleared by Spalteholz's method, showing distribution of carbon in the sternebræ.

FIG. 3.—Anterior extremity of a rabbit, showing the distribution of carbon in the bone marrow. The specimen has been decalcified and cleared by the Spalteholz method.

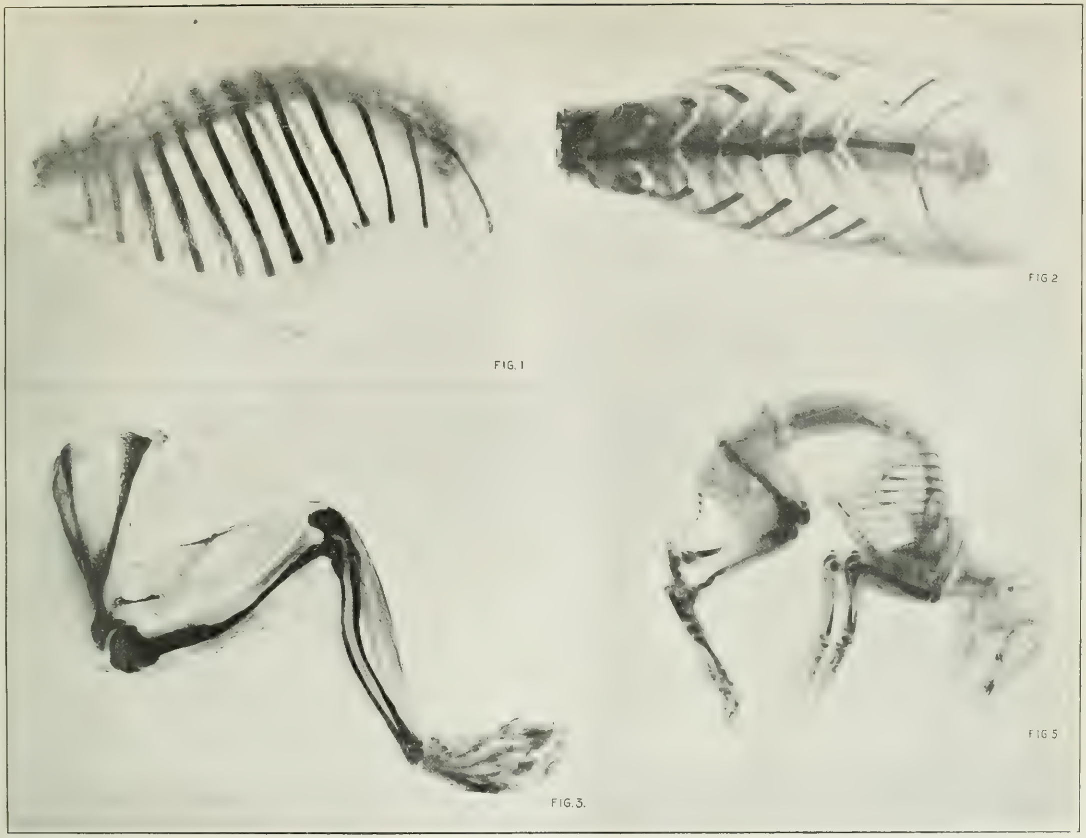
FIG. 4.—View of a decalcified, cleared skull of a rabbit, showing the deposition of carbon granules in the marrow.

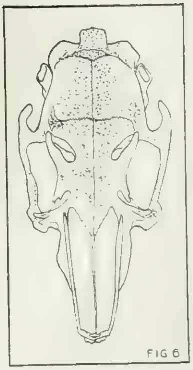
FIG. 5.—Decalcified, cleared rabbit, 3 weeks of age. The animal had received two injections of a carbon suspension (India ink) intravenously and was sacrificed 48 hours after the second injection.

FIG. 6.—Diagram of dorsal view of skull of an adult rabbit. The black dots show the exact distribution of carbon granules in the bone marrow. (The outlines of the bones of the skull in Figs 6, 7, and 8 are after Bensley, "Practical Anatomy of the Rabbit," 1910, and Gerhardt, "Das Kaninchen," 1909.)

FIG. 7.—Diagram of ventral view of a skull of an adult rabbit, the black dots indicating the deposits of carbon in the marrow.

FIG. 8.—Similar diagram of mandible.





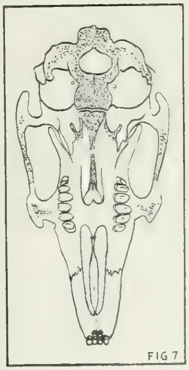


FIG. 4.

THE RESIGNATION OF DR. WILLIAM SYDNEY THAYER

Dr. Frank J. Goodnow, President Johns Hopkins University, Baltimore, Md.

7, April, 1921.

Dear President Goodnow.—Something over three years ago, when the question arose as to my assuming the direction of the medical department, I expressed the opinion that this responsibility might more wisely be entrusted to one who could look forward to a longer tenure of office.

You know, I think, how deep and sympathetic has been my interest in the efforts which the university has been making to improve the standards of medical education and to broaden the opportunities for the study of the art of medicine and of its underlying scientific problems. You are further aware, I am sure, of my unreserved approval and sympathy with those improvements of organization and methods which, in latter years, we have been enabled to initiate through the generous assistance of the General Education Board—improvements which are but the natural evolution from the advances introduced by Osler over thirty years ago.

In the developments which are sure to follow within a few years, it is important that the medical department be under the direction of a man specially qualified by character, training and experience; it is, however, especially desirable that he be one who may reasonably look forward to twenty or twenty-five years of activity in the execution of his ideals and his conceptions.

For this reason I have felt, and more than once have said, that I regarded myself as undertaking these responsibilities for a limited period—until there might appear the men or man obviously qualified for the task.

It is my conviction that this moment has arrived.

I can imagine few opportunities more delightful or congenial or stimulating to the physician of high ideals than those offered by the Professorship of Medicine at The Johns Hopkins University. I am, however, convinced that for the furtherance of the cause of sound medical education and for the development and expansion of those general plans on which we have so happily entered—in the success of which I am deeply concerned—I am convinced that the moment has come to hand over the direction of the department of medicine to a younger man.

I beg leave, therefore, to offer you my resignation as Professor of Medicine at The Johns Hopkins University to take effect on July 1, 1921.

In so doing pray let me assure you that, as in the past, so in the future, my services, in whatever capacity or to whatever extent they may be desired, will always be at the disposition of the university.

Believe me, dear Dr. Goodnow,

Very sincerely yours,

(Signed) W. S. Thayer.

MINUTE ON THE RESIGNATION OF DR. THAYER

In accepting the resignation of Dr. William S. Thayer as Professor of Medicine in The Johns Hopkins Medical School and Physician-in-Chief to The Johns Hopkins Hospital, the members of the boards of trustees of the university and of the hospital, together with the members of the Medical Faculty of the university and of the Medical Board of the hospital, desire to record their deep appreciation of the long and distinguished services rendered to the university and hospital by Dr. Thayer. He began his work as Assistant Resident Physician on Dr. Osler's wards in 1889, and, except for the period of his important war-activities, has continued uninterruptedly in posts of ever higher responsibility until the present time.

In his care of patients in the hospital, in his teaching of students and assistants, in his original studies, especially those on malaria and on diseases of the heart and blood vessels, in his papers and addresses distinguished not only for their contents but also by their clarity, forcefulness and elegance of style, and in his personal relationships with the public, as well as with the profession both in this country and abroad, he has attained an international reputation, and has added notably to the prestige of the hospital which he has so faithfully served; while his rare qualities both of head and of heart make him stand out as the highest type of clinician of his time.

Though Dr. Thayer feels that the direction of the medical clinic should now be assumed by a somewhat younger man, his associates are glad to know that the hospital and university may still avail themselves of his diagnostic skill, his rich experience and his valued counsel.

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EPIDEMIC ENCEPHALITIS

A CLINICAL STUDY

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The recent epidemic of encephalitis began in Europe during the winter of 1916-1917 and the first cases were reported by von Economo[1] from Austria and by Cruchet[2] from Northern France at about the same time. Early the next winter the disease appeared in epidemic form in Paris where it was studied by Netter[3] and others.[4, 5] Following these publications instances of the disease were recognized in many localities throughout Europe. The first cases observed in England were mistaken for botulism,[6, 7] but the error was soon corrected and the identity of the British and continental cases was established as the result of the investigations of McNalty, James, Marinesco and McIntosh,[8] and others.[9, 10]

The malady did not remain confined to Europe, but probably favored in its spread by extensive troop movements, soon appeared in Africa, Asia, Australasia and America.[11, 12] It was apparent, therefore, that the various local epidemics were in reality parts of a great pandemic of a disease of the nervous system.

Since the appearance of the epidemic in the United States during the winter of 1918-1919 we have had an opportunity to observe a large number of patients affected with the disease in the wards of The Johns Hopkins Hospital, and in many instances it has been possible to follow them for a considerable period of time after their discharge. We believe, therefore, that a presentation of the chief clinical features and of the late symptoms and sequelæ of the disease in this series of cases may be of interest at this time.

Nomenclature

The disease, originally described under the headings "Encephalitis lethargica" by von Economo[1] and "Diffuse Encephalomyelitis" by Cruchet[2] in 1917, has recently been given a number of different names, such as Encephalitis myoclonica,[13, 14] Encephalitis choreiformis[15] and Encephalitis comatosa, depending on the predominant clinical symptoms presented by the patient. The tendency to diversity of nomenclature is unfortunate, because the numerous types of the disease so merge one into another that no one name based on the clinical features is applicable to a large number of cases. Thus, Bourges and Marcandier[16] reported an instance of the disease in which choreiform, myoclonic, catatonic, lethargic, athetotic, delirious and comatose stages were observed successively.

Moreover, it is incorrect to combine the term "encephalitis" indicating a pathological process with the descriptive term "lethargica," "choreatica," etc. It would be more logical and more convenient, therefore, to include all cases

of the disease under the heading "Epidemic Encephalitis" and, if necessary for descriptive purposes, to add the modifying phrase "with lethargy," "with myoclonia," etc.

The name "Epidemic Encephalitis" was first employed by Buzzard[17] in 1918 and has lately received wide usage. It is open to two objections which, however, are not serious. In the first place, the lesions in many instances are not limited to the encephalon but may be present in the meninges, spinal cord and peripheral nerves, and it would be more correct to employ the more comprehensive term "meningo-encephalo-myelo-neuritis" in some instances, as proposed by Barker, Cross and Irwin.[18] Unfortunately this designation is too cumbersome for popular usage. In the second place, the term "epidemic" is not applicable to sporadic cases, as Netter has pointed out. Despite these minor objections, the name "Epidemic Encephalitis" will be employed throughout this paper as the most suitable designation so far proposed.

Epidemiology

It is of particular interest that the pandemic of encephalitis occurred almost simultaneously with a pandemic of severe influenza and, as was to be expected, the causal and epidemiological relationship of the two maladies was carefully investigated by a large number of epidemiologists and medical historians. Crookshank[19] concluded, from a study of the historical features of encephalitis and influenza, that epidemics and endemics of a disease similar to the epidemic encephalitis of recent years have been observed for at least four centuries. In great part they have appeared shortly before or after or in geographical proximity to epidemics known variously as "influenza," "la grippe," or "sweating sickness."

The simultaneous occurrence of the two great pandemics in recent years is, therefore, not especially remarkable, for as pointed out above, the two diseases have often occurred in close temporal relationship. Furthermore, although the two pandemics appeared at about the same time, they seem to have been disseminated by somewhat different paths, so that in one part of the world encephalitis preceded influenza, in another influenza preceded encephalitis and in other localities the two occurred simultaneously. Epidemiological investigations of the diseases, therefore, lend little support to the conception of certain authors that epidemic encephalitis is a post-influenzal affection. Moreover, we have as yet no methods of differentiating influenza from certain other acute infections or epidemic encephalitis from similar cases of acute or sub-acute non-suppurative encephalomyelitis. Ophthalmological[20] as well as neurological[21] literature prior to the recent epidemic contains records of cases similar to if not identical both clinically and histopathologically with epidemic encephalitis. A number of these have occurred sporadically. It is probable, therefore, that influenza and epidemic encephalitis frequently occur in sporadic form and that, in consequence, epidemics of either or both of these diseases are possible when conditions at present not well understood are favorable to their wide dissemination.

A study of the records of our cases of epidemic encephalitis has shown that influenza in a recognizable form has seldom preceded directly the onset of encephalitis. In a number of instances, moreover, the patients were born after the major epidemic of influenza. The evidence at hand, therefore, does not favor the assumption that the two diseases are causally related, but since our knowledge of the etiology of each affection is still incomplete, a certain solution of the problem is not possible.

Epidemic encephalitis, although in all probability an infectious disease, is only slightly contagious. It is remarkable that the affection became world-wide in distribution with so few evidences of its direct transmission. Netter could find but eight cases in France and an equal number reported from England in which the disease occurred in more than one member of a family. Although a large number of patients with the disease have been cared for in hospitals, reported instances of bed to bed or even of ward infection are rare. An outbreak in an institution for girls has been reported[8]—the only instance of the kind we have encountered in the literature. Guillain and Lechelle[24] reported a case of the disease which occurred between 15 and 30 days after the patient had visited the home of his father who had died of the disease seven months previously. Roger,[25] in a recent article, stated that, although about 10,000 cases of encephalitis had occurred in France, direct contagion could be traced in only 174 cases. In no locality had a true epidemic occurred. He believed that the disease was transmitted by means of healthy carriers or unrecognized cases.

James,[22] Netter,[23] and Bernard and Renault,[26] studied the epidemiology of a large number of cases in France and in England and concluded that, although widely distributed, the disease occurred chiefly in the more thickly populated districts. The precise mode of dissemination could not be determined from their studies.

In our series of 81 cases we have not encountered a single instance in which more than one member of a family or household was affected, nor has it been possible to determine the mode of infection.

The accompanying chart (Table I) shows the age, sex and color of the patients and also the date of the onset of symptoms. It will be seen that there were 46 males and 35 females. The ages varied from seven weeks to 65 years. Over half of the patients were past the tenth year. The chart shows furthermore that the greatest number of cases developed during the months of January, February and March, although cases appeared throughout the entire year.

Clinical Considerations

It will be convenient to preface the discussion of the symptomatology of epidemic encephalitis by some important inferences which may be drawn from the histopathological studies of the disease.

The wide distribution of the lesions of the nervous system which may be surmised from clinical examination during the course of the affection readily explains the multiplicity and

TABLE I

(THE CASE NUMBER REFERS TO CASES CITED IN THE TEXT)

Case No.	Age	Sex	Color	Date of onset of symptoms	Cerebrospinal fluid				
					Day of disease	Number of cells	Wassermann reaction	Globulin	Gold chloride curve
1	4 years.	M.	W.	January 16, 1920.	90±	10	Negative.	0	0000000000
					102±	3	....	0	1000000000
					160±	5	....	0	0000000000
2	11 years.	M.	W.	February 19, 1920.	3	7	Negative.	0	
					6	28	....	0	
					23	5	....	+	
					45	2	....	+	
					65	1	....	+	1111000000
3	12 years.	M.	W.	October, 1919.	8	10	Negative.	0	0001000000
4	16 years.	F.	W.	November, 1919.					
5	6 years.	F.	W.	November 9, 1919.	4	4	Negative.	+	1121000000
					11	3			
6	65 years.	M.	W.	August, 1919.	60±	200	Negative.	0	0011000000
					62±	90	....	0	
					77±	40	....	0	
7	24 years.	M.	W.	January, 1920.	19	30	Negative.	+	Negative.
					43	17	Negative.	+	Atypical paretic.
8	37 years.	M.	W.	February, 1920.	120	70	Negative.	0	1122210000
9	6 years.	F.	W.	September 16, 1920.	4	14	Negative.	+	
					8	26		+	
10	24 years.	F.	W.	February, 1919.	20	17	Negative.	++	2244211000
11	36 years.	M.	W.	March, 1920.	23	19	Negative.	++	Luetic zone.
					26	44	....	++	Luetic zone.
					49	3	....	+	Luetic zone.
					65	1	....	+	Luetic zone.
12	35 years.	M.	B.	March, 1920.	42	6	Negative.	+	2222200000
13	5 years.	M.	W.	February 4, 1920.	5	55	Negative.	+	1111221000
14	8 years.	M.	W.	May 18, 1920.	1	50	Negative.	+	
					2	40	Negative.	+	1111110000
					3	250	....	+	
					9	66	....	+	
					16	11	....	+	
15	18 months.	F.	W.	September 26, 1920.	4	33	Negative.	0	
16	34 years.	M.	W.	December, 1920.	14	26	Negative.	0	0000000000
17	7 weeks.	F.	W.	April 1, 1919.	5	28	Negative.	+	1122210000
					10	10	....	0	
18	28 years.	F.	B.	May, 1920.	5	9	Negative.	+	
19	19 years.	M.	B.	March, 1920.	4	368	Negative.	0	1444110000
20	2 months.	M.	W.	April 2, 1920.	4	56	Negative.	+	
					7	34	....	+	
					12	22	....	+	
					17	11	....	+	
21	4 months.	F.	W.	March 17, 1920.	1	10	....	0	
					9	20	Negative.	+	0110000000
					17	11	....	+	
					28	8	....	+	
22	5 years.	F.	W.	February 1, 1920.	49	6	Negative.	++	1222100000
23	10 years.	F.	W.	May 15, 1920.	75	28	....	++	
					79	11	Negative.	+	2233443210
					86	6	Negative.	+	0112011100
					95	3	....	+	
24	2 years.	M.	B.	August 8, 1920.	2	18	Negative.	0	
					4	21	....	0	
					7	5	....	0	
					16	3	....	0	
25	37 years.	M.	W.	March, 1920.	21	70	Negative.	+	1222100000
26	25 years.	M.	W.	March, 1920.	120±	3	Negative.	0	0000000000
27	26 years.	M.	W.	June, 1920.	60+	15	Negative.	+	
28	3 years.	F.	W.	September 6, 1920.	6	7	Negative.	0	
					22	6	....	0	

TABLE I—CONTINUED

Case No.	Age	Sex	Color	Date of onset of symptoms	Cerebrospinal fluid				
					Day of disease	Number of cells	Wassermann reaction	Globulin	Gold chloride curve
29	17 years.	F.	B.	February, 1920.	30	225	Negative.	+	1111000000
					35	20	Negative.	+	1111000000
30	27 years.	F.	B.	October, 1920.	5	3	Negative.	0	3442100000
31	42 years.	M.	W.	September, 1919.	30	22	Negative.	+	
32	23 years.	M.	W.	February, 1920.	7	25	Negative.	0	
					10	58	Negative.	0	
					40	4	Negative.	0	
33	3 years.	F.	W.	February, 1920.	120±	3	Negative.	0	1121100000
34	9 years.	M.	W.	February 27, 1920.	180±	Blood.	Negative.		
35	2 years.	M.	B.	March 1, 1920.	27	19	Negative.	+	1111000000
					31	26	....	0	
					34	18	....	0	
					45	26	....	+	
					66	10	....	+	
36	6 years.	M.	W.	February 3, 1920.	90	2	Negative.	+	
37	9 years.	F.	W.	April, 1920.					
38	7 years.	M.	W.	February 19, 1920.	5	36	Negative.	+	1110000000
39	9 years.	M.	W.	February 11, 1920.	8	51	Negative.	+	0110000000
40	29 years.	M.	W.	February, 1920.	?	8	Negative.	0	
41	13 years.	F.	B.	April, 1920.	59	60	Negative.	0	
42	1½ years.	M.	W.	September 20, 1920.	1	8	Negative.	+	
43	2½ months.	M.	W.	June 9, 1920.	6	36	Negative.	+	1111110000
44	5½ years.	F.	W.	May 6, 1920.	60	20	Negative.	+	1111100000
45	2 years.	F.	W.	October 2, 1918.	8	92	Negative.	0	
					17	9	....	0	
46	2 years.	F.	W.	August, 25, 1919.	3	30	Negative.	0	
					6	12	....	0	
47	20 years.	F.	W.	December, 1918.	11	42	Negative.	++	0122110000
					13	70	Negative.	+	1122100000
					16	74	Negative.	0	1143210000
					46	13	Negative.	+++	4555421000
					64	6	Negative.	+	5443210000
48	47 years.	F.	W.	February, 1919.	42	2	Negative.	+	Luetic zone.
					50	7	Negative.	+	Atypical paretic.
49	48 years.	M.	W.	February, 1919.	22	97	Negative.	+++	Meningitic curve.
					27	133	Negative.	++++	Paretic curve.
					52	50	Negative.	+++	Atypical.
					65	3	Negative.	+	
50	33 years.	F.	W.	March, 1919.	7	Blood.	Negative.		
51	32 years.	F.	W.	March, 1919.	60±	4	Negative.	0	0122100000
52	40 years.	M.	B.	July, 1919.	24	40	Negative.	++	2244210000
					38	7	Negative.	+	1223211000
53	37 years.	M.	W.	November, 1919.	35	30	Negative.	0	1121000000
					39	46	Negative.	0	1111100000
					55	10	Negative.	0	0111100000
54	7 years.	F.	W.	February 24, 1920.	120±	3	Negative.	+	0000000000
55	9 years.	M.	W.	January 6, 1920.	360±	38	Negative.	+	0001110000
					390±	6	....	+	4223010000
56	3 years.	M.	W.	May 9, 1920.	7	3	Negative.	+	
					9	3	....	0	
57	8 years.	F.	W.	January 16, 1920.	5	132	Negative.	+	
					7	72	....	+	1122100000
					11	51	....	+	
58	8 years.	M.	W.	June 2, 1919.	4	160	Negative.	0	
					6	250	....	+	
					12	230	....	0	
					17	8	....	0	
59	5 years.	F.	W.	November 27, 1919.	7	27	Negative.	+	0111000000
					17	7	....	+	

TABLE I—Continued

Case No.	Age	Sex	Color	Date of onset of symptoms	Cerebrospinal fluid				
					Day of disease	Number of cells	Wassermann reaction	Globulin	Gold chloride curve
60	6 years.	F.	W.	June 18, 1919.	4	55	Negative.	0	
					5	56	....	0	
61	25 years.	F.	B.	March, 1920.	?	3	Negative.	0	1111200000
62	44 years.	F.	W.	December, 1919.	300+	23	Negative.	+	Negative.
63	48 years.	M.	W.	February, 1920.	330+	5	Negative.	++	Luetic zone.
64	37 years.	M.	W.	December, 1920.	26	23	Negative.	+	0000000000
65	13 years.	F.	W.	December, 1920.	42	3	Negative.	0	
66	19 years.	F.	B.	January, 1920.					
67	20 years.	F.	B.	January, 1920.	60	25	Negative.	+	4411100000
					100	10	Negative.	..	2443311000
68	26 years.	M.	B.	February, 1920.	21	150	Negative.	+	1121000000
					27	150	Negative.	+	2221100000
					37	60	Negative.	0	1222100000
					67	1	Negative.	0	
69	34 years.	M.	B.	February, 1920,	42±	30	Negative.	+	1343210000
					72±	20	....		
70	43 years.	M.	B.	March, 1920.	22	87	Negative.	0	1112210000
					41	18	Negative.	+	
					56	17	Negative.		
71	32 years.	M.	W.	April, 1920.	7	3	Negative.	0	1111100000
72	3 years.	F.	W.	July 17, 1919.	1	2	Negative.	0	1100000000
					3	23	....	+	
					5	59	....	+	
					7	96	....	+	
					12	72	....	+	
73	9 years.	M.	W.	February 8, 1920.	7	1	Negative.	+	1111100000
74	42 years.	M.	W.	January 20, 1921.	19	27	Negative.		
75	13 years.	M.	W.	January, 1921.	11	280			
76	19 years.	M.	W.	December, 1920.	60±	18	Negative.	0	0000000000
77	10 years.	M.	W.	February, 1921.	7	114	Negative.	+	0000000000
78	6 years.	F.	W.	December 15, 1920.	42	5	Negative.	0	1111100000
79	8 years.	M.	W.	February 7, 1921.	4	70	Negative.	+	1111100000
80	5 weeks.	F.	B.	January 28, 1921.	5	270	Negative.	+	
81	8 years.	M.	W.	January 17, 1921.	14	8	Negative.	+	0000000000

diversity of the neurological symptoms encountered. Moreover, these changes are produced by a virus which is capable of damaging functionally important brain areas without necessarily producing permanent anatomical changes (Marinesco [8]). It has been demonstrated, furthermore, both by clinical and histological methods that as one affected area of the nervous system is recovering another area may be attacked, thus adding, in certain instances, new clinical features to an already complicated symptom complex. It is apparent from these considerations, therefore, that the chief characteristic of the malady is the kaleidoscopic appearance of transient neurological symptoms dependent chiefly on alterations in the midbrain and the basal ganglia, and in some instances in the spinal cord and peripheral nerves (Burrows [27]).

In this report no attempt will be made to construct clinical types of the disease depending on the prominence of this or that symptom complex. The important symptoms, however, will be discussed seriatim and illustrated when possible by case reports.

Symptoms and Types of Onset

It is not necessary here to discuss the various symptoms encountered during the early stages of the affection except in a general way, because the sequence of events at onset is clearly set forth in the detailed case histories recorded below.

However, it will be advantageous to call attention to a general division of symptoms at onset which is necessary for a proper understanding of the disease. These manifestations fall into two fairly well defined groups as follows: First, those dependent on a general reaction of the individual to an acute infectious disease; and second, those referable to involvement of the nervous system. These symptoms are fever, headache, malaise, nausea and vomiting, abdominal pain,

irritability and restlessness. In children delirium and convulsions were also frequent at the onset. Drowsiness, insomnia, diplopia and other visual difficulties, involuntary movements, muscular jerkings, tremors, spasticity, root pains, general weakness and psychoses occurred with especial frequency.

The onset may be abrupt or gradual, but in general a period of time elapses between the appearance of the general symptoms of infection and the development of the neurological symptoms.

Disturbances of General Consciousness

Disturbances of general consciousness often combined with psychical disorders have been prominent features of the clinical course in most instances. No constant type of disturbance has been encountered but, on the contrary, all gradations between sleeplessness at one extreme and coma at the other have been observed.

In a few cases sleeplessness of days' or weeks' duration was present and preceding or subsequent lethargic states were not observed. The following case may serve as an example:

Case 1.—W. J., a white boy, aged 4 years, was admitted to the Harriet Lane Home April 23, 1920, because of inability to sleep at night. He had been perfectly well until January 16. That evening he was not able to go to sleep and since that time this condition had persisted. Because of this nocturnal restlessness and activity, he was thought to have chorea. The mother stated that the child stayed awake all night spending the time in constant activity, talking, playing, singing, whistling, etc. Toward morning he would go to sleep and sleep a variable length of time, sometimes all day, sometimes only an hour or so during the entire 24 hours. Various means to combat the insomnia had no effect whatsoever, nor did keeping him awake during the day induce sleep at night.

Paralyses had never been noted. Examination on admission showed a well-nourished boy. No definite abnormal findings were obtained on physical examination. The cerebrospinal fluid was normal. The behavior of the child, however, was very striking. As night-time approached, he became very active, jumping up and down in bed and talking incessantly. He would often stay awake all night, at times talking to himself, at other times whistling and singing, fretting and crying, but never lying absolutely still. He spat a good deal both in the bed and on the floor. He would frequently wet the bed. When excited, he would stammer. The greater part of the night would be spent in ceaseless activity. Toward morning he would fall asleep. As a rule he slept from 3 to 12 hours in the 24; always between 5 a. m. and 6 p. m. The striking point about his sleep in the daytime was that it was very deep and when awakened for meals he would fall asleep immediately, sometimes in the act of eating. It was found very difficult to keep him awake during the morning. On awakening he would behave quite normally until evening when the same behavior would be repeated. Continuous tubs and packs were unsuccessful in bringing on sleep. He was discharged July 11, without any definite improvement in the insomnia. Since his return home there has been but little improvement in his condition.

Again, the insomnia of the acute stage of the disease might be followed by lethargy of several weeks' duration as in the following case:

Case 2.—W. D., a white boy, aged 11 years, was admitted to the Harriet Lane Home February 21, 1920. The past history was uneventful. On February 19, 1920, while at school, he became very excited and talkative and was sent home. His mother noted that he was irrational at times. The following night he became delirious. There had been no convulsions. Examination on admission showed a well-nourished boy, in active delirium, thrashing about in bed. There were no abnormal physical findings. The leucocyte count was 9800. The cerebrospinal fluid was normal (for subsequent examination see C. S. F. chart). The temperature was 100° F. but rose rapidly to 104° F., remaining there for six days, gradually falling to normal on the 15th day, with frequent slight elevations thereafter. He remained in active delirium for seven days, muttering constantly and pulling at the bed clothes. Typhoid fever was suspected, but excluded by the various laboratory examinations. Following the subsidence of the delirious state the patient passed into a state of lethargy in which he remained for ten weeks. At first he could be aroused and made to respond to questions, but he soon passed into deep coma, so that for five weeks it was necessary to administer both food and fluids by gavage. The mask-like facies during this period was striking (Fig. 1). Beyond the somnolence no striking neurological features were made out, until March 12, when external strabismus, a bilateral ankle clonus and a positive Babinski were noted. Hyperpnœa followed with diminished bicarbonate content of the blood serum (26 volumes per cent (Van Slyke)), which was not relieved by sodium bicarbonate. In spite of the administration of 1000-1500 calories per day by gavage, the patient lost considerable weight. From the first of May, he gradually improved, responded to questions, talked and moved his limbs. The ankle clonus, Babinski and strabismus disappeared. The facies remained, however, immobile (Fig. 2). He was allowed to leave his bed and was discharged June 20, apparently well save for weakness and general spasticity. By July he was walking about the house and sleeping well at night. When seen in August, there was noted a slight tremor of the hands and constant clearing of the throat. There were no abnormal movements. He had developed the habit of spitting and was disobedient at times. There was general spasticity and marked stiffness of the carriage in walking. The speech was monotonous (Fig. 3). In September he developed sleeplessness at night, which, though less marked, was still present when seen in December. At this time he seemed fairly well, but was disobedient. He was permitted to go to school.

Sleeplessness at night alternating with deep sleep during the daytime was a characteristic symptom in a few cases during the acute stage and also during the period of convalescence (*v. i.*, under Insomnia).

In other instances drowsiness or sleepiness of a few hours' or a few days' duration often combined with transient diplopia were the only symptoms of a very mild form of the disease.

Case 3.—M. E., aged 12 years, was admitted to the hospital October 30, 1919. He complained of dizziness. He had not had influenza. On October 22, 1919, he had double vision but went to school. He came home and slept all the afternoon. The drowsiness increased steadily until admission. On examination the patient was drowsy but clear when aroused. The legs and arms were somewhat spastic and there was a positive Babinski on the left. The spinal fluid contained 10 lymphocytes per c. mm. The patient slept constantly for four days. Thereafter recovery was rapid and complete.

Case 4.—M. C., a white girl, aged 16 years, was admitted to the hospital November 22, 1919. She had not had influenza. From November 8 to 17, 1919, she felt abnormally sleepy and slept several afternoons. On November 17 she noticed diplopia. This lasted several days, then disappeared, to return again November 21, 1919, for a few hours. The patient returned to school and has remained well (August 30, 1920).

Lethargy was present in many cases and in the severe forms of the disease was usually followed by deep coma.

Case 5.—A. V. F., a white girl, aged 6½ years, was perfectly well until the evening of November 9, 1919, when she complained of slight headache and had fever. The following day she had a generalized convulsion and was unconscious for the remainder of the day. Following this she became stuporous with periods of consciousness, but at all times was difficult to arouse. There were no further convulsions and no vomiting. The patient was admitted to the Harriet Lane Home November 13 on the fifth day of her illness. Examination at this time showed a poorly nourished girl lying in a state of deep stupor. When stimulated she cried out but immediately relapsed into stupor. There were no evidences of paralyses either of cranial nerves or of the extremities. The left arm, however, was held stiffly and there was a tremor of the left hand. The reflexes were active; there was a positive Babinski on both sides and a slight ankle clonus on the left. Examination was otherwise negative except for otitis media on the left. The leucocyte count was 12,000 and the spinal fluid was normal. The Wassermann test was negative with blood and spinal fluid. The child then went into a deep lethargy so that she would not be aroused. She lay perfectly still, making no voluntary movements and was apparently unable to swallow solid food. There was a fine tremor of the tongue and of the hands. The ankle clonus and positive Babinski persisted. At times there was flexibilitas cerea. By December 9 the patient had become brighter and cognizant of her surroundings. The evidences of pyramidal tract involvement had disappeared. By the middle of December she had recovered and was able to sit up. At this time she developed a mild arthritis of the right shoulder and of the right hip. She was discharged January 13, apparently well. Since this time reports from her parents indicate complete recovery without sequelæ.

Case 6.—A. R., a white male, aged 65 years, was admitted to the hospital October 22, 1919, complaining of dizziness and trouble with vision. The present illness began two months before admission with sleepiness and drowsiness which had constantly increased. On October 19, 1919, he felt dizzy and weak and stopped work. On examination his temperature was 103.7° F. and he was comatose. He muttered incoherently. There was bilateral ptosis. The pupils were active and the fundi normal. Coarse muscular jerkings of the arms and legs were observed. The extremities were fairly spastic. The tendon reflexes were normal. The spinal fluid contained 200 cells per c. mm. and a trace of globulin. The temperature remained above 102° F. The patient sweated profusely and emaciated rapidly. He died November 4, 1919.

Autopsy (Figs. 4 and 5).—The brain appeared normal in the gross. Microscopical sections showed marked perivascular infiltration about many of the small vessels in the caudate nucleus, lenticular nucleus, substantia nigra and mid-brain.

Characteristic, then, of the disturbances of general consciousness which may occur in the course of this disease are:

1. The variation of the disturbance from sleeplessness to coma with the intermediate stages which are called apathy, drowsiness, sopor, lethargy, stupor, etc.

2. The long duration of the lethargic state in many instances. A patient may lie for weeks or even months in a state of deep lethargy varying but slightly from day to day or week to week.

3. The fact that many of the patients, although apparently in a state of deep sleep, will respond promptly to a command or reply to a question in a perfectly rational manner, relapsing immediately into the former state of lethargy.

4. The frequent association of a state of general spasticity.

Lethargic states resembling those present in this affection may be observed in a number of acute diseases, such as typhoid fever, and in chronic affections, such as syphilitic basilar meningitis. However, they usually lack the characteristics outlined above, although sometimes the differentiation cannot be made until the patient has been observed over a considerable period of time.

Disorders of psychical activity, when present, are generally combined with some of the disturbances of general consciousness described above. The psychosis in a few instances was the only important symptom and a group of such cases has been reported from this hospital by Hohman.[28] In the majority of our patients, however, abnormal mental reactions, when present, were incidents in the unfolding of the symptom complex of the disease. We shall discuss here only a few of the more frequent mental symptoms encountered during the early stages of the malady. Delirium was infrequent in the adult cases, but was observed often in the early stages of the disease in children (see Case 2).

In the following case there were few symptoms other than delirium and excitement to suggest the diagnosis of epidemic encephalitis:

Case 7.—W. H. E., a white male, aged 24 years, was admitted to the hospital January 27, 1920. The past history was unimportant. The present illness began January 25, 1920, with moderate fever and general malaise. A few days later he had aching of the limbs. Moderate delirium soon appeared, accompanied by visual and auditory hallucinations. The patient was restless and very talkative and confabulation was a striking feature. Transient diplopia, moderate tremor of the hands and slight nystagmus were present. The spinal fluid contained 30 cells per c. mm. The Wassermann test with the blood and spinal fluid was negative. When last seen, January 1, 1921, the patient was mentally clear but restless, talkative and rather expansive. He was also suffering from marked insomnia.

Other patients had irrational periods of variable duration with visual and auditory hallucinations. Occupational delirium was occasionally observed and in rare instances mental reactions not unlike a Korsakoff psychosis were present. One patient worked in the wheat field for days at a time. Another prayed constantly because voices told him "to get right with God." A young colored girl knew someone was hiding in the room waiting to kill her. Spontaneous, unprovoked outbursts of laughing or crying were present in several of our patients. These attacks were not provoked by adequate emotional stimuli and must be looked upon as abnormal. It is of interest that many of these patients had a mask-like facies and bilateral rigidity of slight extent (pseudo-bulbar syndrome).

It is not necessary at this time to detail all the mental symptoms encountered; a full discussion of this phase will be found in the papers by Abrahamson,[29] Hohman[28] and others.[30]

Ocular Disturbances

Disturbances of innervation of the extra-ocular muscles were often present during the active stage of the disease. *Diplopia* was frequently the first symptom referable to involvement of the nervous system noticed by the patient and in some instances preceded the appearance of any other symptoms by many days. The characteristic feature of these palsies was their transient and variable character. Thus, in

some cases diplopia was present on one examination and absent the next, or an internal strabismus, present for a short time, was followed by an external strabismus of variable duration. Furthermore, although most of the muscles innervated by the third nerve might be palsied successively, it was rare indeed to observe involvement of all of them at the same time.

Ptosis was frequent, usually bilateral, although often more marked on one side. It was seldom complete. It frequently contributed considerably in the production of the expressionless facies of the patients. In some instances it was impossible to decide whether the slight bilateral ptosis was dependent on faulty innervation or on lethargy, for drooping of the upper lids occurs in the somnolent state.[31]

Disturbances of associated eye movements were comparatively rare. One patient could not rotate the globes above the horizontal plane. A few had weakness on lateral deviation. Inability to converge was common, although usually transient.

We encountered a few instances of paralysis of accommodation. These patients complained of inability to see near objects clearly and to read fine print. In such cases we assumed that true paralysis of accommodation was present, unless there were optic nerve changes or conspicuous disorders of innervation of the extra-ocular muscles.

Nystagmus was present in a large number of cases and was of two types. In the first, it was similar to that observed in *early multiple sclerosis*, viz., the common rhythmical oscillations with a quick and slow phase. In the second, the eyes seemed as if held in place by springs, and attempts at fixation produced wide, irregular oscillatory movements of the globes in all planes, including the *anterio-posterior*, until the eyes came to rest, often in good alignment. The characteristic quick and slow phases were absent in such cases. Rarely, attacks of coarse jerking of the globes occurred without obvious provocation. So far as we know, this phenomenon has not been described in any other disease. Sicard and Kudelski[32] gave the name ocular myoclonia to this type of nystagmus.

Ophthalmoscopic examinations were made repeatedly in most cases. Hyperæmia of the discs was occasionally present. Optic neuritis with swelling of the discs was observed in the following case and also in Case 23 below:

Case 8.—E. F., male, white, aged 37 years, was admitted to the hospital July 29, 1920, complaining of headache. The present illness began February 1 with dizziness and weakness. About March 1 walking became difficult and he had pain in the back. For several days there was incontinence of urine. Frontal and temporal headache was constant; the left arm became weak and there was some dimness of vision. He noticed a few muscular jerkings. Examination showed a mask-like face with slight bilateral ptosis. The left optic disc was raised and hyperæmic; the edges were indistinct. The right disc was normal. There were no muscular jerkings. The spinal fluid contained 70 lymphocytes per c. mm. and a trace of globulin. There was no fever at any time. The patient was often irrational at night. He improved slightly and left the hospital against advice August 16, 1920.

Disorders of Motility

Disorders of motility were present in the majority of our cases. They were so diverse in character that it is impossible to classify them in well-defined groups. For descriptive purposes, however, we shall group all the motor phenomena under three heads as follows:

1. Disorders of tonicity.
2. Paralyses and Pareses.
3. Hyperkinetic phenomena.

1. *Disorders or Tonicity.*—At the onset of the malady general spasticity associated with other evidences of meningeal irritation, the so-called meningismus, may be present, as in the following case:

Case 9.—K. R., a white girl, aged 6 years, was admitted to the Harriet Lane Home September 19, 1920. She had been well until September 16 (three days before admission) when she complained of headache and vomiting. Throughout the day she was nauseated, drowsy and had no appetite. Toward evening her neck became stiff and a physician who was called regarded the condition as due to meningitis. The patient soon became unconscious but there were periods when she could be aroused. Examination on admission showed a poorly nourished white girl lying in a stuporous state with head retracted and back somewhat stiff. When spoken to she opened her eyes, relapsing immediately into stupor. There was retraction of the neck and apparently pain on flexion. There was no evidence of paralysis, the reflexes were active and there was a suggestive Kernig sign. The leucocyte count was 12,000; the spinal fluid showed 14 cells per c. mm. and a faintly positive globulin reaction. The temperature was normal. The Wassermann test was negative with the blood and spinal fluid. The evidences of meningeal irritation quickly passed away and by September 21 the patient had come out of her stuporous state and appeared bright. A second lumbar puncture done on September 23 showed 26 cells and a positive globulin reaction. The patient was discharged September 30 and has remained well since that time.

Later, however, evidences of disturbance of the pyramidal or extra-pyramidal motor tracts may appear. The former will be discussed with the paralytic phenomena and we shall therefore confine our attention here to those disorders of tonicity which may not be associated with evidences of pyramidal tract involvement.

(a) *Hypotonicity,* associated with ataxia or weakness of the extremities, was present in the following instance:

Case 10.—R. W., a white woman, aged 24 years, entered the hospital in March, 1919, complaining of jerking of the eyes and inability to stand. In October, 1918, she had had a febrile disease, called influenza, which had kept her in bed a week. February 23, 1919, she had severe headache and dimness of vision. Her eyeballs and eyelids were in constant motion. She felt poorly and went to bed, where she remained until admission to the hospital. About two weeks after the onset her legs seemed stiff and she could not walk. Her jaws jerked violently so that speaking was difficult. Since March 24, 1919, she had been unable to stand alone or to walk. Examination showed a well-nourished woman, mentally clear, not drowsy. There were frequent spasmodic contractions of the jaw and eyelids. The eyeballs jerked violently in all directions, both spontaneously and on attempts at fixation. Upward deviation was limited. There was slight bilateral ptosis. The speech was thick and scanning. The right abdominal reflex was not obtained. The muscles were markedly hypotonic and the movements were inco-ordinate. The spinal fluid contained 17 cells per c. mm. and a considerable amount of globulin.

The Wassermann test with the blood and spinal fluid was negative. There was a slight elevation of temperature during the first two weeks. At intervals the patient was drowsy; at other times there were attacks of unprovoked laughing and crying. By July 5, 1919, the jerking of the jaws had ceased and the jerking of the eyeballs was less marked. The incoördination had improved considerably and the patient could walk a few steps alone. It is possible that the hypotonicity, incoördination, scanning speech, nystagmus and motor weakness were dependent on a cerebellar localization of the process, but of this we have no definite proof. However, the resemblance to certain cerebellar syndromes is striking.

(b) *Hypertonicity,* often widespread, was a more frequent symptom. It was present in all the severe or fatal cases and often associated with waxy flexibility, as in the following instances:

Case 11.—C. B., a white man, aged 36 years, was admitted to the hospital in March, 1920. The past history was unimportant. The present illness began March 12, 1920, with fever and dizziness. The family noticed that the patient was restless, euphoric and talkative. He complained of pain in the elbows and general tremor. On March 29 his speech was thick and there was inability to swallow. There was double vision. When first examined March 30, 1920, the patient was well oriented but depressed. There was marked tachypnœa and attacks of "smothering." The pupils were unequal. There was slight nystagmus. Dysarthria was present and the patient was unable to swallow. The face was mask-like. There was slight strabismus. The tendon reflexes were normal. There was very marked general rigidity with waxy flexibility. The patient remained in about the same condition for nine months. Incontinence of urine and feces was present at all times. The rigidity persisted and was of so high degree that he was practically incapable of voluntary motion. He was fed by gavage. The face was expressionless and speech was absent or markedly dysarthric. At times there were attacks of fever, often associated with tachycardia and marked tachypnœa and hyperpnœa of several days' duration. The respiratory rate was 50 per minute for several days. The patient at present (after a year) is a rigid vegetating organism incapable of voluntary motion or of continuous thought.

Case 12.—C. D., a colored man, aged 35 years, complained of difficulty of vision of four weeks' duration. The present illness began March 27, 1920, with pain over the heart which lasted a day. The next day he felt giddy and sleepy but continued to work. That evening he had diplopia and was told his eyes were crossed. On examination the face was expressionless and mask-like. There was bilateral ptosis with weakness of both external recti. There was paralysis of accommodation. There was a coarse, irregular nystagmus and tremor of the lips and tongue. Coarse tremor of the arms was also present. The tendon reflexes were active. There was slight catatonia. The blood and urine were normal. The cerebrospinal fluid contained six cells per c. mm. and a trace of globulin. The Wassermann test was negative with the blood and spinal fluid. The lethargy increased and generalized spasticity appeared. The facial muscles became weak and transient dysphagia appeared. The patient was clear mentally until a few days before death. Rhythmical to and fro movement of a hand, arm or leg, and myoclonic movements of muscle groups were frequently observed. There was no fever. Lethargy increased to coma and the patient died two months after the onset of symptoms.

In other instances hypertonicity occurred in association with a coarse Parkinsonian tremor and a mask-like facies—the so-called Parkinsonian type of the disease. This syndrome occurred both in adults and children * and was often present for a considerable period of time. Indeed, Souques, Moreau and Pichon [33, 34] state that they have observed instances of epidemic encephalitis followed by true paralysis agitans. However, this observation needs confirmation, for these are the only cases that we could find in which the syndrome persisted. Even in these a sufficient period of time has not elapsed to form a definite conclusion.

* See Case 2 above.

2. *Paralyses and Pareses.*—Paralyses of upper or lower motor neuron type may be present during the active stage of the disease.

(a) Monoplegias, hemiplegias, paraplegias or diplegias associated with exaggerated tendon reflexes and other evidences of pyramidal tract involvement may occur. In most instances they were of short duration, followed by complete recovery. The following cases illustrate this type of motor disturbance:

Case 13.—C. B., a white boy, aged 5½ years, had been well until February 6, 1920, when he complained of headache and had fever. The following day he seemed better but that afternoon he vomited and had a general convulsion. Following this he became drowsy and the convulsions became more numerous, occurring every few minutes. He was admitted to the Harriet Lane Home February 9. Examination at this time showed a fairly well-nourished boy lying in a state of coma. Convulsions similar to the type described occurred at five to ten minute intervals. Examination was otherwise negative. These convulsive attacks became less frequent and ceased February 11. The following day he regained consciousness and performed voluntary movements. There seemed to be weakness in the left hand, but otherwise no paralysis was noted. The spinal fluid on admission showed 55 cells per c. mm. and a positive globulin reaction. The temperature was about 101° F. for the first three days after admission and gradually fell to normal. The patient improved, became bright and co-operative. The weakness of the left arm, however, persisted. He was discharged March 14. When seen in September, the patient was quite well and the weakness in the left arm had entirely disappeared.

Case 14.—M. J., a white boy, aged 8½ years, had been well until May 1, 1920, when he complained of slight headache and of burning of his eyes. The evening of May 18 he behaved queerly. He seemed dazed and would not respond to questions and the mother noted twitching of the right side of the mouth; he rapidly became unconscious and was brought to the Harriet Lane Home. Examination at that time showed a well-nourished boy lying in a stuporous state, from which he could be aroused at times in response to stimulation. Involuntary movements were noted which were almost entirely of the left side. The temperature was 100° F. but rose rapidly to 105° F., and then gradually fell, becoming normal on the sixth day after admission. The leucocyte count was 19,000. The spinal fluid showed 50 cells per c. mm. and a positive globulin reaction. The Wassermann test was negative with the blood and spinal fluid. There were no further convulsions but shortly after admission a right hemiplegia was noted. This, however, was transient and on the following day there were no evidences of weakness. On this day the child roused from his stupor, seemed bright and mentally clear. He was discharged perfectly well June 2. When seen three months later he showed no evidence of his former illness.

Case 15.—R. A. G., a white girl, aged 1½ years, had developed normally and was perfectly well until September 17, 1920. At that time she became irritable and a few days later the mother noticed that the child was feverish. On September 24 she vomited and the fever continued, reaching 103° F. On September 26 the child could not walk because of stiffness of the right leg. The left leg became involved the following day. This spasticity relaxed during

sleep, but even then spasmodic contractions were noted which were severe enough to awaken the child. *Examination on admission* showed a well-nourished female infant, conscious and very irritable. The legs were extended and markedly spastic, and passive motion of the limbs evidently caused pain. There was a peculiar brawny condition of the skin and subcutaneous tissue. The deep reflexes were hyperactive, the Babinski and Kernig signs were negative. The upper extremities were unaffected and there was no evidence of cranial nerve involvement. The temperature was 101° F., the leucocyte count was 8500 and the spinal fluid showed 33 cells per c. mm. and a negative globulin reaction. There was no incontinence. This spasticity and general irritability became less marked by October 2 and the patient was able to walk with assistance. She was discharged and in two weeks was walking in a normal manner. At this time restlessness and wakefulness at night were noted. The child would sleep well until midnight but during the remainder of the night was wakeful. This wakefulness was not relieved by the administration of chloral hydrate. Gradually, however, this condition subsided and at the present time the child sleeps well and there are no evidences of residual paralysis.

(b) *Cranial nerve palsies* were present at some time in the majority of cases during the active stage of the disease. As a rule they were transient or recurrent and referable to involvement chiefly of the third, fourth, sixth and seventh cranial nerves or their tracts. Since these have been described above, we shall group together here the disorders of motility dependent on faulty innervation of the facial, masticatory and bulbar muscles.

The mask-like facies was so frequent that the diagnosis without that symptom was hazardous. The photographs (Figs. 1, 2, 3, 6, 7, 8, 10) show clearly this striking feature. The cause of the phenomenon is not clear. It was associated constantly with defective mimicry.

Dysarthria was present in a number of cases. As a rule, the patients complained that the tongue felt thick and the face stiff. In a few instances it was dependent on twitching of the muscles of the tongue. Dysphagia was often combined with the dysarthria as in Cases 11 and 26 and the following case, No. 16:

Case 16.—W. D., a white male, aged 24 years, was admitted to the hospital December 31, 1920, complaining of giddiness. In October, 1920, he had an acute febrile illness for three days. The present illness began rather suddenly December 17 with roaring in the left ear. On the following day he had intermittent attacks of pain down the left arm and behind the left ear. December 21 he had diplopia and "thickness of the tongue." He also noticed that he could not hear well. Drowsiness became marked and there were frequent painful jerkings of the left arm. He also developed difficulty in swallowing and a nasal voice. Difficulty in starting urination soon appeared. Examination showed a drowsy and dull patient with a mask-like face. He was clear when aroused but was partially deaf. The left pupil was larger than the right and there was slight nystagmus. Both optic discs were slightly hyperæmic. There were a few coarse non-rhythmical fascicular twitchings of the muscles of the face and limbs. The reflexes were normal except for a positive Babinski on the left. Mastication and swallowing were possible but were performed slowly and carefully. There was marked disturbance of pronunciation of the labials and linguals. He complained of numbness of the face and tongue. His temperature was 102° F., but in four days reached normal. The spinal fluid contained 26 lymphocytes per c. mm., and a slight increase of globulin; the gold chloride curve was normal. The Wassermann test was negative with the blood and spinal fluid.

Unilateral and bilateral facial weakness was frequent and often transient. The following case is a good example:

Case 17.—M. H., a white infant, aged 7 weeks, was admitted to the Harriet Lane Home April 4, 1919. She was born at term and had been considered normal until the first of April when she became dull and drowsy. Following this she could not be roused; there was slight fever. Examination on admission showed a well-nourished infant in coma. When stimulated she became spastic, but when relaxed had the appearance of a sleeping infant. Examination showed right facial weakness, bilateral ptosis, and slight general spasticity (Fig. 6). The temperature was 101° F. on admission but fell within 24 hours to normal. The leucocyte count was 8500 and the spinal fluid showed 28 cells per c. mm. and a faintly positive globulin reaction. The child improved and became less drowsy and the facial weakness and spasticity disappeared. She was discharged May 3, 1919, and did well at home. She died in June following an acute illness, the details of which were not obtainable.

Trismus, associated with tender masseters, was present in the following instance:

Case 18.—M. W., a colored woman, aged 28 years, was admitted to the hospital May 14, 1920. Four days previously her mouth and tongue had become very sore on attemps to move them and she was unable to open her mouth on account of pain. On examination the temperature was 99° F. but soon reached 102.5° F. The masseters were very tender on pressure and the patient could not open her mouth. She was drowsy and there was dysarthria. There was a double external strabismus which developed under observation. There was incontinence. The cerebrospinal fluid contained 5 cells per c. mm. and a trace of globulin. The reflexes were slightly hyperactive. The Wassermann test was negative. May 15, 1920, the fever reached 103.5° F. and the patient died suddenly.

Tachycardia was occasionally observed and in some instances was possibly referable to involvement of the vagus.

Hyperpnœa was present in six of our cases and deserves special mention. In each of these this symptom occurred during the early stages of the disease and was associated with a diminished bicarbonate content of the blood plasma. However, in several instances it was not relieved by the administration of sodium bicarbonate. The hyperpnœa was therefore not the result of acidosis but dependent on disturbance of the central respiratory regulatory mechanism. Brief records of patients manifesting this symptom may be of interest.

Case 19.—J. C., a colored man, aged 19 years, was admitted to the hospital March 4, 1920. The patient's mother stated that "he sees double and talks out of his head." The present illness began March 1, 1920, with staggering following a slight injury to the head. The next day the patient was irrational, weak and drowsy, and complained of double vision. On examination he was very lethargic and talked irrationally. When aroused he was rational and talked in a low monotonous tone. The face was mask-like (Fig. 7). There was bilateral ptosis with slight internal strabismus. There were many coarse jerkings of the muscles of arms, legs and abdominal wall. The reflexes were normal. The leucocyte count was 11,400. The cerebrospinal fluid contained 368 cells per c. mm. The globulin test was negative. The Wassermann test was negative with the blood and spinal fluid. The incoördination of the eye movements, slight nystagmus and fascicular twitchings persisted for many days. The temperature, which was 104° F. on admission, gradually returned to normal. The tachycardia and tachypnœa persisted; the respirations were usually about 36 to the minute. Lethargy diminished gradually. April 4, 1920, the patient was found in collapse. The respirations were very deep and more than 60 per minute. The pulse was rapid.

The CO_2 tension of alveolar air was 15 mm. Hg.; the bicarbonate content of the plasma was 40 volumes per cent (Van Slyke). Morphine gr. $\frac{1}{4}$ was given and this was repeated in one hour. The respirations fell to about the normal rate and the tachypnœa did not return. The patient gradually recovered and when last seen nine months after the onset of the disease was well except for a peculiar stiffness of carriage, an expressionless facies, and a few tic-like movements of the face.

CASE 20.—A. W., a white boy, aged 8 weeks, was admitted to the Harriet Lane Home April 16, 1920. He had been born at term and breast fed up to the onset of the present illness which had begun eight days before admission when it was noted he did not nurse well and had fever. Four days before admission he had a convulsion, which was followed during the next few days by a series of similar attacks, generalized in character. He refused to nurse. On admission to the hospital, examination showed a poorly nourished infant lying quietly in bed; there was marked hyperpnœa and general muscular rigidity. There was a transient ptosis of the right eyelid and hyperactive reflexes. Otherwise examination was negative. The temperature was normal. The leucocyte count was 22,000 and the spinal fluid showed 56 cells per c. mm. and a positive globulin reaction. The bicarbonate content of the blood was 28 volumes per cent (Van Slyke). There were no evidences of tetany. It was necessary to feed the child by gavage for the first 24 hours. In spite of the administration of sodium bicarbonate to the point of rendering the urine alkaline, the hyperpnœa continued. By April 20 this symptom had disappeared and the child was discharged in good condition except for slight spasticity. When seen November 30 he had completely recovered. (See also Cases 2 and 11.)

CASE 21.—A. Y., a white infant, aged 4 months, had been well until March 17 when following her bath she vomited and had a generalized convulsion. Following this she became unconscious and an internal strabismus was noted. She was admitted to the Harriet Lane Home the same day. Examination on admission showed a well-nourished female infant lying in an apathetic state; she could be aroused, however, and was rather fretful. There was definite hyperpnœa. A slight internal strabismus and ptosis of the left eyelid were noted; otherwise the examination was negative. The spinal fluid was normal. The bicarbonate content of the blood serum was 19 volumes per cent (Van Slyke). There was no acetone in the breath (Higgins). The urine was normal save for a few white cells and the phenolsulphonephthalein excretion was 65 per cent in two and a half hours. There were no evidences of tetany. On account of the hyperpnœa and the diminished bicarbonate content, sodium bicarbonate was administered by mouth to the point of rendering the urine alkaline, without effect on the hyperpnœa which continued when the bicarbonate content reached 48 volumes per cent (Van Slyke). Evidently then the acidosis was secondary to the hyperpnœa which was probably due to stimulation of the medullary center. The patient became less drowsy but the internal strabismus and ptosis persisted, and a slight facial weakness appeared. The hyperpnœa varied during the following three weeks; sometimes it was well marked, at other times slight. Several courses of sodium bicarbonate were given without effect. The spinal fluid on March 26 showed 20 mononuclear cells per c. mm., and a positive globulin reaction. On April 14 the child seemed brighter; the hyperpnœa, the ptosis and facial weakness had disappeared. The spinal fluid returned to normal and she was discharged with slight internal strabismus on April 28. She has since been entirely well.

(c) *Symmetrical paralysis* of peripheral nerve type was observed in six cases. These were characterized by a gradual onset, extensive flaccid paralysis of upper and lower extremities, more marked in the distal portions, slight sensory disturbances, muscular atrophy, and usually slow but eventual recovery.

CASE 22.—C. J., a white girl, aged 5 years, had been perfectly well until the onset of her present illness February 1, 1920, when she awakened complaining of aching in her knees. The following day the child appeared perfectly well but that night again complained of aching in her knees. On the third day her parents noted that the child walked stiffly. This stiffness increased and weakness of the legs developed to such a degree that three weeks after the onset the patient was unable to walk. No swelling or tenderness of the knees developed. The patient then complained of headache and about one week before admission weakness in the hands was noted. Beyond slight irritability, there was no change in the patient's mental condition. She was admitted to the hospital on March 25, seven weeks after the onset of her illness. Examination at that time showed a well-nourished white girl who was unable to stand, was quite irritable, but well oriented and apparently normal mentally. There was neither facial nor ocular paralysis but a general muscular weakness of upper and lower extremities. The legs were moved but little even on stimulation. There was some atrophy but this was not marked. The grasp of both hands was weak. There was ataxia. No disturbance of sensation could be demonstrated. The deep reflexes were absent at elbows, knees and ankles. The spinal fluid was under normal pressure with 6 cells per c. mm. and gave a strongly positive globulin reaction. The Wassermann test was negative with the blood and spinal fluid. The patient remained in the hospital one month. During that time the muscular weakness became noticeably less marked. This muscular weakness was symmetrical and more marked distally than proximally. The deep reflexes remained absent and there was never any sensory change. At the time of discharge, April 24, the patient could move her arms and legs well and could stand with assistance. By the middle of July she had recovered strength in her limbs and was able to walk with support. By September she was able to run and play with apparent control of her extremities, and by the last of November seemed completely recovered.

The other causes of symmetrical paralysis of lower motor neuron type were excluded in this case and there remained a widespread paralysis of upper and lower extremities developing rather suddenly with complete recovery.

CASE 23.—F. S., a white girl, aged 10 years, was brought to the Harriet Lane Home July 30, 1920, because of weakness. She had been well up to May 1, 1920. At that time she fell. A week after this she complained of headache and vomited. This was repeated several times. There was no fever. Her appetite became poor and on June 1 weakness in her legs was noted, at first only after walking. Subsequently this increased until she was unable to walk, and about the middle of July weakness was noted in the arms as well. There was no visual disturbance. Examination on admission showed a poorly nourished child with general muscular weakness. She was unable to walk without assistance and after making a few steps complained of feeling very tired. There were no cranial nerve palsies. The eyes reacted to light and on accommodation. There was a definite optic neuritis more marked on the right. The legs showed generalized muscular weakness with some muscular atrophy. The arms showed muscular weakness to a less extent with a very weak grip. The deep reflexes were all absent. The superficial reflexes were present. The spinal fluid was under slightly increased pressure with 28 mononuclear cells per c. mm. and a strongly positive globulin reaction. The Wassermann test was negative with the blood and spinal fluid. There was no disturbance of sensation, nor had the patient complained of any sensory disturbance. The child remained in the hospital until August 19. During this time her condition improved. The appetite became better. The optic neuritis became slightly less marked and the spinal fluid returned gradually to normal (see c. s. f. chart). The weakness of the arms and legs became definitely less, so that the patient was able to sit up without assistance and to walk a few steps. The knee kicks were still absent but the arm reflexes returned. There was a slight foot drop and some muscular atrophy particularly in the

hand. The galvanic stimulation of muscles of the legs gave the reaction of degeneration. This was not present in the muscles of the forearm. The patient was seen again September 22 and had improved during her month at home. She had been sitting up in bed but had not been allowed to walk. There was definite improvement in muscular strength and control, and she was able to sew. Ophthalmological examination showed a mild optic neuritis. There was an ataxic tremor of the hands with atrophy but no wrist drop; ataxia of the legs with definite improvement in muscular power. The deep reflexes were still absent. A letter from the mother on December 22, 1920, stated that the child was steadily improving, walking around the house without tiring and sleeping well at night.

In summary, an acute polyneuritis in a previously well girl with evidences of increased intracranial pressure, inflammatory reaction in the spinal fluid, optic neuritis, generalized muscular weakness of lower motor neuron type, more marked in the legs; absence of sensory disturbance, with recovery within seven months.

Case 75.—A white boy, aged 13 years, was admitted to The Johns Hopkins Hospital February 15, 1921, complaining of soreness in his back and legs. His past history was negative. The present illness began January 1, 1921, with pain while chewing. The next day he had tingling of his fingers, and a few days later numbness, pain and weakness of the legs developed. About that time he also had severe headache and pain in his back. Examination showed a well-developed boy. The fundi and ocular movements were normal. There was slight general atrophy of the muscles of the hands, feet, arms and legs. The muscles were tender on pressure. There was weakness of the muscles of the extremities and back and slight weakness of the muscles of mastication. Sensation was intact on objective testing. There was hyperæsthesia over the feet. There was no fever. The blood counts were normal. The Wassermann test with the blood was negative. The spinal fluid on the 11th day of the disease contained 280 cells per c. mm. and gave a positive reaction for globulin. The muscles of the legs and arms gave either complete or partial reaction of degeneration. The urine contained a few leukocytes. The boy left the hospital February 23, 1921 much improved. The tenderness on pressure had disappeared and he could rise to a sitting posture which was impossible on admission. By the middle of March the muscle strength had returned to such a degree that he could walk alone.

Case 25.—D. F., a white man, aged 37 years, was admitted to the hospital April 2, 1920. He complained of vomiting and numbness of the feet. The present illness began the middle of March, 1920, with "aching all over" especially of the legs and head. He felt feverish and had a chilly sensation. These symptoms lasted about a week, when repeated nausea and vomiting appeared. He became so weak that walking was impossible. On examination the patient was drowsy and answered slowly but accurately. The face was expressionless. There was slight nystagmus on upward deviation. The pupils were active and the fundi were normal. There were weakness and atrophy of the muscles of the feet, legs, hands and arms. Sensation for touch, pain and temperature and recognition of position was diminished below the knees and elbows. The knee jerks were sluggish; the ankle jerks were not obtained. The tendon reflexes of the elbows were present. The leucocyte count was 2840. The cerebrospinal fluid contained 70 cells per c. mm., with a trace of globulin. The Wassermann test was negative with the blood and cerebrospinal fluid. While the patient was in the hospital there was no fever. The calf and arm muscles wasted rapidly and the reaction of degeneration was present. The patient left the hospital against advice and died six days later.

Case 26.—A white man, aged 25 years, was admitted to The Johns Hopkins Hospital August 20, 1920, complaining of inability to walk. His present illness began in March, 1920, when he noticed that his hands were numb. He began to have pains in his arms, legs and eyes. Seven weeks before admission he had fever and chilly sensations. Three weeks later he had difficulty in speaking and trembling of the limbs and face. He soon developed hiccup, great weakness and difficulty in swallowing. He vomited frequently. On examination he showed poor memory and disorientation for time. The facial muscles were weak and deglutition and articulation were very difficult. The eyes were normal. The tendon reflexes were exaggerated and there was an equivocal Babinski sign. The muscles of the extremities were weak and there were numerous and widespread fibrillary twitchings. Reaction of degeneration was present in some muscles of the calves and arms. The leucocyte count was 6200. The cerebrospinal fluid contained 3 cells per c. mm. and gave a negative globulin reaction. Except for the rather abrupt onset the resemblance to progressive muscular atrophy was striking. However, when the patient was last seen, the fibrillary twitchings and bulbar palsy had disappeared and the muscles had attained nearly normal size and strength.

3. *Hyperkinetic Phenomena.*—Under this heading we shall describe a group of symptoms referable to neuromuscular hyperactivity which we have observed in patients with epidemic encephalitis. Recent medical literature contains many reports of cases presenting various types of hyperkinesias.[35] In some of these such names as "encephalitis myoclonica" and "encephalitis choreiformis" were employed to designate the disease type. In others the close resemblance of the involuntary movements to those observed in Sydenham's chorea,[15, 38, 39, 40] in Parkinson's disease,[33, 41] in Friedreich's myoclonia[27] and in other less well-known maladies,[36] has been pointed out. However, until the pathology of these phenomena is better known, it is not desirable to attempt to divide encephalitis into types depending on the character of the hyperkinesias. Furthermore, our knowledge of the pathological alterations in the large group of affections characterized by the presence of hyperkinetic phenomena is still too meager to allow completely satisfactory correlation of the clinical symptoms and structural alterations.

The hyperkinetic phenomena encountered during the active stages of encephalitis consist of non-rhythmical muscular twitchings, fibrillary twitchings, tremors, choreiform and athetotic movements, myoclonias and certain rhythmical spasmodic movements. In some instances, furthermore, convulsions may occur.

(a) *Muscular twitchings* were present in the majority of the adult cases, rarely in children. They may be fibrillary, fascicular or involve a whole muscle or group of muscles. In many instances only the muscles about the mouth or in the eyelids were affected, but in other cases the twitchings were widespread. The movements were quick, usually nonrhythmical and often violent. At times they were accompanied by pain. They differed from the more rhythmical myoclonias and also from the usual fine fibrillary tremors.

(b) In one case observed by us (Case 26) *true fibrillary tremors* of the muscles of the limbs and of the intrinsic muscles of the hands with widespread muscular atrophy were present. This patient also presented many other symptoms commonly observed in progressive muscular atrophy such as dysphagia, dysphonia and signs referable to pyramidal tract involvement, but it is fairly certain that the picture was pro-

duced by the virus of epidemic encephalitis. In the following case, also, fibrillary twitchings were observed:

CASE 27.—W. B., a white man, aged 26 years, was admitted to the hospital complaining of burning of the eyes. The present illness began three months before admission with "aching all over," shooting pains in the joints, burning of the eyes, and painful micturition. There were also photophobia and difficulty of vision. On examination the patient was drowsy and the face was expressionless. There was slight nystagmus. An occasional quick jerk of the right arm was observed. The legs were stiff and painful on passive motion. The arms were ataxic. The tendon reflexes were normal. There was numerous fibrillary twitchings of all the muscles of the extremities, especially of the interossei. The face was paræsthetic. The temperature occasionally reached 99.5° F. The spinal fluid contained 15 cells per c. mm. and a trace of globulin. The Wassermann tests were negative.

(c) *Tremor* was frequent, especially in patients with slight general spasticity. As a rule, it was rather coarse and fairly regular, but unlike that observed in paralysis agitans. In other instances fine tremor of the fingers was present. Types of tremor accentuated by voluntary movement (intention tremor), however, were not observed by us.

(d) *Myoclonia,* viz., rapid, rhythmical contractions of a muscle or group of muscles, with or without movement at the joints, was observed in a large number of cases. In some instances a rapid to and fro movement of a whole segment was produced by the involvement of synergistic groups of muscles. As a rule, the attacks of myoclonia were spontaneous, but in some instances they were precipitated by voluntary motion. Case 11, recorded above, affords a good example of this type of motor disorder, as does the following:

CASE 28.—E. T., a white girl, aged 3 years, was admitted to the Harriet Lane Home September 9, 1920. Previously the child had been well except for tantrums of crying and wanting her own way, which she had had for about a year. One month before admission she vomited and during the month this was repeated several times. On September 6 the child seemed sleepy and on the following day had fever and was unable to sit up. She remained drowsy but could be aroused. On September 8 twitchings about the mouth and slight tremor of the left hand were observed, which became more marked up to the time of her admission. Speech had become less distinct. There had been none of the temper tantrums since the onset of the present illness. Examination on admission showed a well-nourished girl lying in a state of semi-stupor, occasionally rising to a sitting position. There was constant twitching of the left side of the face and occasionally the muscles of the left side of the neck and deltoid and upper arm would twitch also. When offered an object, the child would grasp it with the right hand. There was general spasticity, more marked on the left. The deep reflexes were present, there was no clonus. The leucocyte count was 7000 per c. mm. and the temperature never went above 100° F. The spinal fluid was normal. The child remained in the hospital until September 30. During the first part of her stay the twitchings of the left face, arm and to a less extent of the leg were marked. A few days later, however, it was noted that the twitching was present also on the right side. There was no actual paralysis of any part. These muscular twitchings never involved all the muscles of an extremity at one time, but there would be twitchings of parts of one muscle group now here, now there. These movements resembled the muscular twitchings produced by a weak galvanic current. By September 30 they had entirely stopped and the patient was discharged apparently recovered except that she refused to grasp objects with her left hand. This condition was still present when she was last seen (January 6, 1921). There were no other sequelæ.

(e) *Choreiform movements* indistinguishable from those of acute chorea were present in a few cases. The following record demonstrates clearly the difficulties of diagnosis in such instances:

CASE 29.—K. B., a colored woman, aged 17 years, was admitted to the hospital March 23, 1920, complaining of jerky movements and pain in the neck. The patient was delivered by normal labor February 14, 1920. Nine days later she had sharp shooting pains in the limbs and neck. The pains recurred about every ten minutes and were very severe. She felt very weak. On March 18, 1920, the patient was delirious and her temperature had reached 105° F. Marked jerking of the arms, legs and body appeared. There was diplopia. Examination revealed violent, generalized, purposeless movements, indistinguishable from those in acute chorea. There was delirium with visual and auditory hallucinations. The patient was sure someone was in the room waiting to kill her. There was slight ptosis on the right. The spinal fluid contained 225 cells per c. mm. and a trace of globulin. Her temperature was 103° F. but slowly fell to normal. The violent choreiform movements gradually disappeared and the patient became lethargic. Succeeding the chorea extensive myoclonia was observed. The attacks of the latter occurred spontaneously or were precipitated by voluntary movement. They occurred in various groups of muscles. Following these, quick fascicular jerks were observed. The ptosis was variable—often bilateral. The face was expressionless and rigid and masseter tenderness was present (Fig. 8). From time to time sharp pains were present in the extremities. Marked internal strabismus appeared associated with coarse jerky movements of the globes in all directions, even anteroposteriorly. These were usually caused by attempts at fixation but were occasionally spontaneous. There was also coarse intention tremor of the arms. The symptoms gradually disappeared and when last examined eight months after onset the patient had practically recovered. The eyes still oscillated slightly and there was some strabismus.

(f) In the following instance peculiar movements consisting of *bilateral rhythmical tonic spasms* were observed:

CASE 30.—M. G., a colored woman, aged 27 years, entered the hospital October 5, 1920, unable to talk on account of peculiar motor phenomena described below. Her illness began October 1 with vomiting. In a few hours she was irrational and delirious. On examination the face was smooth and expressionless. There was slight nystagmus. The optic discs were very red, but the margins were sharp. About ten times every minute the patient had a tonic spasm consisting of bilateral contraction of the neck, abdominal and limb muscles. The contraction produced flexion at the neck, elbows, wrists, hips, knees and ankles. These spasms gradually became less severe and ceased. There was marked sweating, at first limited to the face but eventually involving the whole body. The patient was lethargic at intervals. Recovery was uneventful and complete.

The striking characteristic of these tonic spasms, whether limited to an extremity or generalized, is their rhythmicity. In the case just recorded they occurred every 5-6 seconds during one period of observation. Later they became less frequent but still remarkably rhythmical. In another instance only the left leg was involved and the spasm occurred every 3-4 seconds. Marie and Levy have given the name "bradycinesia" to this type of motor disorder.

(g) *Hiccup* of long duration, usually occurring in attacks alternating with free intervals, was present in the following instance:

Case 31.—A white man, aged 42 years, was admitted to the hospital October 6, 1919, complaining of hiccup and depression. The present illness began in September, 1919, with an attack of hiccup of three days' duration. Then there was a period of freedom for a week when the symptoms reappeared. The patient also felt weak and unsteady on his legs. He had a fine tremor of the hands and constant hiccup when first examined.

It is of interest that during the Canadian epidemic [44] of encephalitis a number of instances of continuous hiccup were observed. Their relationship to encephalitis is still obscure, for apparently no other symptoms referable to the nervous system involvement were observed. At the present time a widespread epidemic in which hiccup is the only symptom is present in the eastern part of the United States and in Austria.[45] Siccard [46] and others [43, 47] have called attention to the prevalence of epidemic hiccup in France and its relation to abdominal and diaphragmatic myoclonia.

(h) *Convulsions* were frequent at the onset in the young but occurred in only one instance in an adult. Case 13 illustrates a frequent form of onset of the disease in children.

A few generalizations may be drawn from the study of the hyperkinetic phenomena. First, a variety of movements may be present in one case. Thus choreiform movements may be replaced by myoclonia and this in turn by muscular twitchings. Secondly, none of these, with the possible exception of ocular myoclonia described above, is of much diagnostic importance when considered alone. However, a history of the sequence of events will usually direct attention to the probable diagnosis. Thirdly, a variety of disorders of motility have been observed as late symptoms or sequelæ of the malady. These will be discussed below.

Disturbances of Sensation

The sensory disorders encountered during the active stages of epidemic encephalitis are of considerable interest, although not frequent. In the early stage of the disease general hyperæsthesia, dependent in all probability upon meningeal irritation, is not uncommon. It is usually very transient. Occasionally also marked tenderness of the masseters may be demonstrated.* In one patient this phenomenon was so marked that chewing was impossible and the mouth could not be opened. The patient was given anti-tetanic serum, although a few hours later diplopia and strabismus developed.

Headache is a frequent symptom and although usually present only for a few days at the onset, may persist for weeks or months. In the later instances it may be considered as a direct neurological symptom and not as a part of the general reaction to the toxins.

Special interest is attached to a type of radiating pain, occasionally constant but more often paroxysmal in character, which resembles closely the familiar root pains of tumors of the cord or the lancinating pains of tabes dorsalis. This type of pain was usually present in those patients who later developed marked muscular twitchings or choreiform or myoclonic movements. The following case record is typical:

Case 32.—W. H., a white man, aged 23 years, was admitted to the hospital March 1, 1920, complaining of nervousness and drooping of the eyelids. He had had a mild attack of influenza in September, 1918. The present illness began February 22, 1920, with severe intermittent pain in the left ring finger which radiated upwards to the elbow. The pain occurred every 10-15 seconds and lasted in all two days. Following this he felt shaky and had a severe generalized tremor. Chewing was difficult. The speech was jerky. Drowsiness and diplopia appeared and the patient noticed that his eyelids drooped. On examination the temperature was 102° F. The face was expressionless and there was bilateral ptosis. The pupils were active. There was a coarse rapid tremor of the hands. The extremities were quite spastic. The tendon reflexes were very active and there were bilateral patellar and ankle clonus. The cerebrospinal fluid showed 25 cells per c. mm. and the globulin reaction was negative. The Wassermann test was negative with the blood and cerebrospinal fluid. The fever soon disappeared. The pains in the arms became less severe. The left forearm muscles became weak and the arm reflexes were increased on that side. The pain down the left arm was the last symptom to disappear. When last seen, eight months later, the patient felt much better, but had developed insomnia at night with drowsiness during the daytime. He was able to continue his medical studies.

In another patient the radicular pains in the limbs were followed by marked atrophy of the distal muscles of all the extremities associated with extensive fibrillary twitchings.

Numbness or paræsthesia of one-half of the body, of an arm, a leg or of the face, was occasionally complained of by the patient. Objective sensory disturbances, however, were seldom demonstrated in such instances.

The polyneuritic form of the affection, discussed above, may occur with sensory changes similar in all respects to those encountered in an alcoholic polyneuritis. In other cases of this form of the affection no sensory changes could be demonstrated.

Miscellaneous Symptoms

Sweating is often a prominent feature of the disease. In one instance (Case 29) it was limited to the face for several days but later became general. The gowns of some patients were constantly drenched with sweat, although they had little or no fever at the time (see Case 6).

Urinary incontinence was present in a few instances. As a rule the patients were comatose or very lethargic, but in other cases transient loss of sphincter control was probably dependent on involvement of the mechanism controlling the bladder and rectum.

Late Symptoms and Sequelæ

It might be expected that a disease characterized histopathologically by inflammatory alterations widely distributed throughout the nervous system would produce, after the cessation of the acute process, changes in the delicate regulatory mechanisms which manifest themselves in various psychic and neuromuscular disorders. Such, indeed, is the case. But the

* First called to our attention by Dr. H. M. Thomas.

chronicity of the disease, the development of new symptoms after apparent recovery and the rapid changes that may occur, lead us to believe that in many instances these apparent sequelæ are in reality evidences of a disease process still active.

The phenomena, described below, may appear at any time during the course of the disease. As a rule, however, they occur after the subsidence of the acute stage or after apparently complete recovery.

(1) *Psychic disorders* are present during the period of convalescence in a large number of cases. They consist of mental depression, a feeling of unreality and inferiority, changes in disposition, irritability, increased psychomotor activity, disobedience in children, inability to concentrate and often marked mental fatigue. Occasionally delusional and confusional psychoses may appear. Emotional instability consisting in uncontrollable laughing or crying is not uncommon. In some instances these mental residuals are not striking when the patient is in the ward. They appear, however, as soon as he returns to his home or his business. These unfortunates, usually aware of their abnormalities, often develop marked neurasthenic and psychasthenic states. The papers of Bramwell,[48] Claude,[49] Bergé and Hufnagel,[50] Papen *et al.*,[51] McNalty,[8] Abrahamson,[29] Leahy and Sands[52] and others may be consulted for interesting details of this phase of encephalitis.

(2) *Insomnia* as a late symptom or sequel of the affection is so characteristic, especially in children, that it will be discussed in some detail. During the active stage of the disease sleeplessness is often present, especially in instances of the disease in which hyperkinetic features were noted (McNalty,[8] Ellis,[53] McAlpine,[54] Sicard,[55] Boyd,[44] Laporte and Rouzaud,[56] Roger,[57] Dimitz,[15] Neal,[58] Abrahamson,[29] Findlay and Shiskin[59] and others). During the later stages, often some time after apparent recovery, a syndrome described by one of us in a previous report[60] may make its appearance.* This consists of sleeplessness at night alternating with marked drowsiness or even lethargy during the daytime which manifested itself as follows:

Towards night the parents note a change in the child's behavior; he becomes restless, excited and runs about the house prying into things. He will not obey. When put to bed he does not go to sleep but jumps up and down, shakes the bed, calls frequently to his parents, talks incessantly, sings, blows, whistles and spits on the floor. He becomes destructive, tears the linen, shouts, runs out of his room and when confined will not remain quiet. He spends the whole night in constant activity. He is able to recognize his parents but makes queer sounds and strange gestures. He frequently soils the bed, plays with his feces or masturbates. At dawn he falls asleep and is difficult to arouse. This sleep usually lasts until noon. When awakened during this sleeping period, he is stupid and falls asleep again during a conversation or examination. For a few hours in the afternoon he seems fairly normal, but at dusk the restless, sleepless period begins again. We have observed this symptom-complex in 12 children (and in a mild form in two adults), a remarkably high percentage of the total cases. The striking features of the syndrome are the similarity of behavior of the affected children and the chronicity of the symptoms. In none of them has a return to normal taken place (February, 1921), although improvement has been noted in some.

The symptom-complex may follow closely "recovery" from a typical acute attack, or develop late in convalescence; or it may even appear as the only recognizable clinical feature of the disease. It may not be as severe as outlined above, but be manifested as a mild disturbance in sleep of brief duration. Illustrative cases are as follows (see also Cases 1, 2, 7, 15 and 32 above):

Case 33.—M. F. G., a white girl, aged 3½ years, had been well until late in February, 1920. At that time she complained of feeling tired and her parents would notice that frequently during the day she would lie down and sleep for a while and then get up and play again. She would do this from three to five times during the day. About the 1st of May she became restless at night and would not go to sleep before early in the morning. Bromides were ineffective. Towards the middle of May her mother noticed weakness of her legs and jerky movements of the arm when grasping objects. No strabismus or facial weakness had been noticed. She was admitted to the Harriet Lane Home for observation June 12, 1920. Examination at that time showed a small poorly nourished child, thrashing about restlessly in bed and quite irritable. However, she seemed bright mentally. Physical examination was negative except that the patient was disinclined to stand, although she would use her legs fairly well in bed. The deep reflexes were obtained with difficulty. The child did not sleep until after midnight. The spinal fluid was normal. A letter from the father December 22 says that the patient has greatly improved except that she does not always sleep well at night. However, she sleeps better than she did previously. She will occasionally drop off to sleep during the daytime. She was active and mentally normal.

Case 34.—H. G., a white boy, aged 9½ years, had been previously well until February 27, 1920, on which night he was unable to go to sleep, was restless and had a peculiar staring expression. Since that time he had not slept well at night. A facial paralysis was noted by the mother four days after the onset. Up to the time of admission he had been going to sleep about 4 a. m., sleeping often until noon. He was admitted for observation July 13, 1920. Examination at that time showed a rather thin boy, somewhat apprehensive, with a definite left facial paresis (Fig. 9). The pupils were unequal, but reacted to light. There was definite internal strabismus and hyperactive reflexes. The spinal fluid was contaminated with blood. During the night in the hospital the patient slept very little. He was seen again in September, somewhat improved but he still remained awake most of the night, during which time he was excitable, in constant motion, wanting to do something all the time, but generally rational when spoken to. The left facial weakness was still present but very slight. He was said to be not as bright as he had been before his illness. No abnormal muscular movements had been observed, but at times there was increased salivation. His voice was monotonous and his expression blank and worried. A communication from his mother, December 27, stated that there was very little improvement. At times he seemed better, at others he relapsed into insomnia.

Case 35.—C. J., a colored boy, aged 1¾ years, had developed normally and had been well up to March 1, 1920, when he had fever and was fretful. The fever subsided in a few days but toward the last of the month the child became very lethargic, sleeping most

* This syndrome has since been observed in various European countries. (See J. Am. M. Ass. 1921, LXXVI, 934.)

of the time. He refused food and seemed to have some difficulty in swallowing. There were no convulsions and he vomited only once, which was at the beginning of the illness. Examination on admission March 27, 1920, showed a well-nourished colored boy lying in a state of lethargy from which it was difficult to arouse him. The facial expression was quite blank with a flattening of the nasolabial fold suggesting a "mask-like facies" (Fig. 10). However, there was no paralysis present. The extremities were freely moved and the reflexes were active. The temperature was 100° F. The leucocyte count was 6000. The spinal fluid on admission showed 19 cells per c. mm. and a positive globulin reaction. During the day the patient would at times sit up in bed and occasionally stand. There was a ptosis of the eyelids and a lack of attention to his surroundings. His facial expression is well illustrated in the accompanying photographs. The patient had frequent attacks of naso-pharyngitis with elevations of temperature but gradually became brighter, took more notice of things and was discharged in good condition May 20. The following month he was admitted for tonsillectomy and appeared normal (Fig. 11). He did well until September 20 when he was wakeful and restless all night, tossing about in bed. Following this he did not sleep well at night, waking up generally around 11 o'clock, screaming and pointing at imaginary objects. Sometimes he would stay awake all night and at other times he would go back to sleep after a couple of hours of wakefulness. Occasionally during the waking period waving movements of the arms were noted. This condition gradually improved and when last seen, January 6, 1921, he was sleeping better, although he woke up every night for an hour or two. However, he was less irritable and excited than before. He had developed enuresis and the mother had recently noted an increased amount of saliva with drooling particularly at night.

Case 36.—H. W., a white girl, aged 9 years, had been previously well except for the ordinary exanthemata until March, 1920, when she had an acute attack of scarlet fever. She recovered from this and in April a tonsillectomy was performed. It was observed at this time that the child was restless at night and during the month of April jerky inco-ordinate movements were noted and certain tic-like movements such as constant pulling at her neck. From this time wakefulness had persisted. She generally slept four to six hours in the twenty-four, usually between 4 a. m. and 10 a. m. She gradually became more excited at night so that she was admitted to a private sanitarium on June 13, 1920. There was no history of paralysis or ocular symptoms. Physical examination at that time was entirely negative. During the day the child was bright and cheerful. As night came on, she became excited, over-active, laughing loudly at times, at others talking incoherently, standing up in bed, pulling at her neck, whistling and singing. She usually went to sleep about 3 a. m. The patient was placed in an ideal environment in a quiet place in the country with a special day and night nurse. However, there has been no material change in her condition and up to the present time she still suffers from inability to sleep at night.

Case 37.—B. J., a white boy, aged 6 years, had been perfectly well until February 3, 1920, on which day it was noted that he was restless and delirious. This excitable state lasted for three days and nights. During this time purposeless movements of the hands and arms were observed which had disappeared by February 20. At this time also a tremor of the hands was noted and a change in speech, the child talking much more slowly than formerly. No paralyses were noted, but inability to sleep, at night, developed. He was admitted to the Harriet Lane Home May 3, 1920. Examination at this time showed a well-nourished boy, apparently of normal mentality; his speech was slow and scanning and there was a coarse tremor of the hands. The cerebrospinal fluid showed 2 cells per c. mm. and a positive globulin reaction. The Wassermann test was negative with the blood and spinal fluid. The child remained in the hospital until May 31. During this time he slept fairly well at night, but the tremor of the hands and the slow scanning speech persisted. When seen July 15 this speech defect had entirely disappeared and the patient talked in a normal manner. He was bright and responded readily to questions. The tremor had also disappeared, but the insomnia had become worse, the patient usually getting to sleep at 2 or 3 a. m. and sleeping until noon the following day. This condition improved so that by September he was sleeping fairly well at night. A report from the parents stated that the child died the middle of November following an acute abdominal condition, the nature of which was not clearly understood. No autopsy was performed.

2. *Neuromuscular phenomena* of various types may appear during the period of convalescence and are rather characteristic of the disease. In general these consist of muscular twitchings or jerkings, tremors, choreiform and athetoid movements, tics, general psychomotor overactivity, spasticity and rarely paralyses (Buzzard and Greenfield,[61] Marie and Levy,[62] Sicard,[63] Netter,[64] Claude,[49] Papin *et al.*,[51] Lortat-Jacob and Hallez,[65] Kahn,[66] McNalty,[8] Leahy and Sands[52] and others).

A state of general psychomotor overactivity, characterized by restlessness, is often present. The patients seem unable to remain still; they must be doing something constantly and are always hurrying. Simple acts are performed with exaggerated movements. In one patient, an adult, this condition has been present for nearly eight months but is gradually disappearing. In children, due to lack of conscious control, this abnormal state is even more marked.

Tic-like movements are commonly present and have been described by various authors.[67, 68, 15] They consist in the frequent repetition of some act and are indistinguishable from the common tics of psychasthenics. The following cases illustrate these movements:

Case 38.—C. S., a white boy, aged 7 years, was admitted to the Harriet Lane Home February 24, 1920. He had been perfectly well up to February 19, at which time it was noted that he talked strangely. That night delirium, restlessness and muscular twitchings were noted. The following day the restlessness continued and a physician who was called thought the boy had chorea. After a sedative was given, the movements stopped, and the boy became stuporous. On admission to the hospital he was in stupor and slept a great deal of the time. However, he could be aroused and would respond to questions. There was no paralysis and the physical examination was practically negative. The temperature on admission was 103° F. It dropped in the course of five days to normal. The leucocyte count was 16,000 and the spinal fluid contained 36 cells per c. mm. and gave a positive globulin reaction. There were no choreiform movements observed in the hospital. The patient gradually recovered from his stupor and was discharged April 5, 1920. A few days after his return home he became restless at night and up to the present time (nine months) has not slept well at night. According to the description of the mother, he would stay awake at night talking to himself. He generally went to sleep about 4 a. m. and slept until noon. He became very emotional and shy. Shortly after discharge various tic-like movements made their appearance. Thus when he was seen in May he would repeatedly touch his forefinger to his tongue and place the wet finger to his cheek and also on various objects of furniture. He was very nervous and in constant activity. Since his illness his disposition had changed. He was afraid of everybody and had become morose and queer. His memory had become impaired so that he did not remember having been in the hospital. This condition has persisted except that other tic-like movements have followed, replacing the earlier ones, such as licking the back of his hand and wiping his forehead, making beckoning movements with fingers, snapping his

fingers, making circular swinging movements of his arms, going about the room rapping on various articles of furniture, etc. In July he began to spit and when seen was constantly expectorating on the floor so that at the end of an hour there was quite a collection of fluid. His speech became more indistinct and mumbling. When seen December 20, there was no improvement in his condition, he still remained awake and active all night and it was necessary to place him in an institution.

CASE 39.—P. E., a white boy, aged 9½ years, was well until February 11, 1920. On that night he slept poorly. Two days later he had double vision and was at times delirious. Insomnia followed and he was admitted to the Harriet Lane Home February 19, 1920. Examination on admission showed a well-nourished boy, at times rational but at other times threshing about in bed in active delirium. The pupils were dilated but no palsies were made out. The reflexes were active. The leucocyte count was 12,600. The spinal fluid showed 51 cells per c. mm. and gave a positive globulin reaction. The Wassermann test was negative with the blood and spinal fluid. During the periods of delirium the patient made exaggerated uncoördinated movements of the limbs and sedatives were required. The temperature on admission was 101° F. and fell to normal on the fourth day. At this time the delirium and movements had stopped, the patient was mentally clear and co-operative, slept well at night, and the physical examination was entirely negative. He was discharged March 5. Almost immediately after his return home he developed wakefulness at night and a series of tic-like motor phenomena, present both night and day. These consisted in, first, "stretching" motions of the arms, in which the arm was extended and several quick motions made as one does in stretching. A similar movement was made with the legs. When asked why he did this, he said he felt better after the motion had been made. There was no marked pain in the extremities. Second, the forefinger and middle finger were raised to the nose and a wiping movement made, the patient blowing through the nose at the same time. There was no nasal discharge. Third, constant movements of the fingers, as placing the middle finger on the forefinger, the ring finger on the middle finger and the little finger on the ring finger. Fourth, the patient would take a deep breath and make a quick expiration as one does in clearing the throat. However, there was no production of mucus or sputum. The boy talked in a whisper and was very fidgety and excited. Mentally he was clear and there was no return of the delirium. Physical examination was negative. The spinal fluid was normal except for the presence of globulin. He was kept in the hospital several days, and on account of his activity and inability to sleep was transferred to the psychiatric ward. He remained there for several weeks without marked improvement. When seen at home in July these movements were still present and he had developed the habit of spitting. In September the arm motions had stopped but the insomnia had not improved. By the last of October he was sleeping fairly well at night, though waking up frequently. No tic-like movements were observed and he had stopped spitting. However, he was very nervous and excitable, crying easily and extremely fidgety, constantly using his hands to handle objects on the desk, to fumble with his clothing, etc. He was sent to school and has done fairly well.

The characteristic features of these movements are, first, their constant repetition, and second, their short duration. Thus, after one tic-like movement has vanished, another movement or habit spasm appears in its place. These movements are especially marked at night in many instances.

General spasticity as a late symptom or sequel of the disease occasionally associated with a coarse tremor or with choreiform or athetoid movements was present in a number of our cases. There is often a peculiar stiffness of the carriage, the arms, back and shoulders scarcely moving and the steps very short. The face may be expressionless and the voice monotonous, as in Case 2 above.

Hemiathetosis was present in the following case:

CASE 40.—W. S., a white man, aged 29 years, complained of jerking of the left arm and left leg. In February, 1920, the patient had a febrile disease said to be influenza. Following this he was very drowsy and went to sleep while driving or eating. Subsequently there was a period of insomnia. About the middle of April, 1920, the left arm became weak; then the left arm, left leg and left side of the face began to jerk. On examination the patient was mentally clear. There were continuous choreo-athetoid movements of the left arm, left leg and left face without changes in the reflexes or tonicity. The hyperkinetic phenomena were gradually becoming less marked when the patient was last seen (November, 1920).

Paralyses of long duration were rarely observed. In the following instance, however, the paralysis present for many months was rapidly cured by persuasion and was therefore of hysterical origin. In another patient (Case 28) the weakness of the left hand was organic.

CASE 41.—H. H., a colored girl, aged 13 years, stated that in March, 1920, she had measles. On April 1, 1920, she began to have headaches accompanied by nausea and vomiting. She was very drowsy—sleeping constantly for a week. On examination the patient was drowsy and listless and the face was expressionless. There was slight fever. The spinal fluid contained 60 lymphocytes per c. mm. The patient was discharged in June, 1920, apparently well. A week later her right arm became rigid and paralyzed and very tender to the touch. After this condition had persisted for five months, the patient was again admitted to the hospital. The right arm was held rigidly and was very hyperæsthetic. The sensory disturbance was distributed in a "shirt sleeve" area. The condition was believed to be hysterical and was quickly cured by suggestion.

Salivation.—Increased production of saliva and frequent spitting were often observed as late symptoms of the disease in children. One child while being examined would spit to such an extent that there was quite a collection of water on the floor at the end of a half-hour. These children are usually trained by their parents to expectorate into a handkerchief and it was not unusual to see a child coming into the clinic holding a saliva-soaked handkerchief in his hand and constantly spitting into it. In our experience it usually comes on late in convalescence and is frequently more marked at night than in the daytime. At first we were inclined to attribute this habit to the peculiar mental attitude of these children, but it seems more likely that an actual increased production of saliva is present. Netter [72] has called attention to this symptom and makes it the basis of his pilocarpin treatment. Bergé and Hofnagel,[50] Pierre Marie and Levy,[69] and Leahy and Sands [52] report salivation in several cases and Babonneix [70] and Netter [71] acute parotitis (not epidemic) developing in the course of the disease.

It is interesting in this connection to recall Gordon's paper [73] on an epidemic in 1913 of a peculiar malady characterized by enlargement of the salivary glands, salivation, nervous symptoms and spinal fluid changes. The condition, he stated, was not mumps.

Enuresis nocturna was frequently observed in children following recovery from this disease. These children drink a

great deal of water, especially at night during the waking period.

Laboratory Examinations

The leucocyte count according to various authors [18, 48, 58, 73, 74] is usually normal or slightly increased. The average given is between 10,000 and 14,000 per c. mm. The differential formula is usually not altered, unless there is a leucocytosis, and then there is a polymorphonuclear increase.

It should be mentioned that the variability of the leucocyte count may depend somewhat on the stage of the disease when the count is made. The erythrocyte count and the hemoglobin content were not altered in uncomplicated cases. The leucocyte count in our cases varied between 2600 and 20,000. In general, however, the leucocyte count was normal or slightly increased.

Spinal Fluid.—The earlier reports of the disease stated that abnormalities of the cerebrospinal fluid were infrequently observed.[1, 3] Later studies, however, have demonstrated that alterations of the spinal fluid are usually present during the acute stage of the disease. Benard [75] reviewed this subject in 1920 and from his studies and those of other authors [74, 48, 15, 68] the following description of the cerebrospinal fluid in epidemic encephalitis is taken:

(1) The fluid is clear and colorless.

(2) The pressure is normal or slightly increased.

(3) There is a cellular increase early in the disease, which rapidly disappears, although Netter [76] stated that the pleocytosis may reappear during a relapse. The cells are usually mononuclear in type. Polymorphonuclear cells may be present in a small percentage. The total cell count varies from normal to 200 or more per cubic millimeter, averaging from 25 to 40.

(4) The amount of globulin is normal or increased. The increase in the amount of globulin, however, is generally not as marked as the increase in the number of cells (" dissociation cytoalbuminique " (Bernard [75])); an important point in differential diagnosis.

Table I above shows the results of the examinations of the cerebrospinal fluid in our cases. This table brings out the following points:

(1) The fluid from cases during the acute stage of the disease practically always shows some change, either (a) increase in cells, or (b) increase in globulin, or (c) both.

(2) The cell count at the onset may be only slightly increased, rise quickly to a peak and then fall rapidly. For this reason several examinations of the fluid are of more value than a single examination in showing abnormalities, and especially in differentiating from tuberculous meningitis (*v. i.*).

(3) The globulin content is usually only slightly increased except in cases showing evidence of cord involvement; these may show strong globulin reactions. This increase may persist for months after recovery.

(4) The gold chloride curve reveals nothing of special diagnostic value (see Table I).

Urine.—The examination of the urine reveals nothing of diagnostic value. The findings are those of any acute infectious disease.

Bacteriology.—Various observers * have reported the isolation both ante and post mortem of a variety of organisms with which they claim to have reproduced pathologic changes in animals similar to those found in human epidemic encephalitis. These organisms fall into four groups: First, Gram-positive cocci; second, Gram-negative cocci; third, filter-passing globoid bodies, and fourth, a filtrable virus (bacteria-free). If each of these produce similar lesions in animals on intracerebral injection, then one must conclude either that they are all different forms of the same organism, or that the lesions reproduced are non-specific. This uncertainty, together with the negative findings of so many competent observers, make us feel that, although the bacterial nature

* Von Wiessner [78] in 1917 in Vienna isolated a Gram-positive diplostreptococcus with which he produced pathologic changes in animals. This organism was also found by von Economo [35] in the spinal fluid, blood and nasal secretions of patients. Dimitz [15] found the same organism during life and post mortem in cases of the choreiform type of the disease, as did Cohn and Lauber [79] in Munich. The latter were unable to reproduce the disease in animals. Maggiora, Montovani and Tombalato [80] found a small Gram-positive diplococcus, which was agglutinated in a dilution of 1: 100 by the patients' serum and by the serum of convalescents from the disease but not by normal serum. They were able to produce lesions in guinea-pigs on inoculation with cultures of this organism. Maggiore and Sindoni [81] isolated from the blood and spinal fluid of five patients a Gram-negative coccus which on passage through rabbits produced the pathologic picture of encephalitis. They liken these cocci to those isolated by Noguchi in poliomyelitis. Ottolenghi and his co-workers [82] were able to produce pathologic changes in guinea-pigs with a filtrable virus obtained from the blood, spinal fluid and nasal washings. Gabri [83] cultivated an organism (M. tetragenus) from the blood in three cases, but his animal inoculations proved negative. Boccolari and Panini [84] found a Gram-negative diplococcus in the blood. Marinesco [8] in England saw in stained sections Gram-negative diplococci and Gram-positive bacilli, but considered their etiological relationship to the disease open to question. McIntosh and Turnbull [85] and more recently McIntosh [86] obtained positive inoculations in monkeys and rabbits with a virus from the brain of a fatal case, but were unable to grow an organism.

Bradford, Bashford and Wilson [87, 88, 89, 90, 91] recovered from the brains of cases of encephalitis and polyneuritis a filtrable virus, and Basher, Caldwell and Coombie [92] a Gram-positive organism.

In America Stafford [93] and Morse and Crump [94] recovered Gram-positive cocci and the latter succeeded in producing lethargic states in rabbits after intra-cerebral injections. Loewe, Hirschfeld and Strauss [95] isolated a minute filtrable organism from the brain, cerebrospinal fluid and nasal washings, which on injection into rabbits and monkeys produced pathologic changes. Thalheimer [96] recovered a similar organism. House [97] recovered a Gram-positive diplococcus from the brain in a fatal case and Dunn and Heagey [98] a green streptococcus from the blood. Levaditi and Harvier,[99] in France, obtained a filtrable virus from the brain of a fatal case which they claimed reproduced in rabbits typical lesions of epidemic encephalitis. This virus could be passed through several rabbits and then become pathogenic for guinea-pigs. However, the virus was not neutralized by the sera of patients convalescing from the disease. Amoss [100] found that the blood serum of recently convalescent cases of epidemic encephalitis does not neutralize the virus of poliomyelitis, whereas the serum of convalescent cases of poliomyelitis possesses this neutralizing power.

of epidemic encephalitis is highly probable, the causative organism has not yet been definitely demonstrated.

Diagnosis

The clinical features of this disease are so polymorphous that the diagnosis is often particularly difficult even in the presence of an epidemic. At best it is an exclusion diagnosis, for a large number of affections of the nervous system may produce symptom complexes similar to those observed in epidemic encephalitis. Only by elimination of all other known sources of nerve cell damage and by observation over a considerable period of time can the diagnosis be made with any degree of certainty.

The most important diseases which may simulate epidemic encephalitis are the various other types of encephalitis, tuberculous meningitis, abscess and tumor of the brain, acute poliomyelitis and syphilis of the nervous system.

Four types of non-purulent encephalitis are encountered with sufficient frequency to be of importance in differential diagnosis.

(a) Acute non-suppurative encephalitis following the acute infectious diseases such as typhoid fever, measles, mumps, scarlatina and pertussis, is not uncommon, especially in children. In most instances the history of the preceding illness, the presence of numerous convulsions, the short duration and the absence of prolonged lethargy and ocular palsies help in their differentiation. In a few instances the two diseases may be indistinguishable clinically and the diagnosis must rest on the history of the preceding illness. The following case is an example:

Case 42.—W. E., a white boy, aged 6 years, was taken ill April 18, 1920, with cough, coryza and conjunctivitis. The following day an eruption appeared. The description of this eruption suggested measles. He did fairly well until the night of April 22 when he had several convulsions. He was admitted the following day to the Harriet Lane Home. Examination at this time showed a well-nourished boy, conscious, lying quietly in bed. There was a faded morbilliform eruption over the face, body and extremities. Examination was otherwise negative. Lumbar puncture showed a clear fluid with 130 mononuclear cells per c. mm. and a positive globulin reaction. No tubercle bacilli were found in the fluid. At first the patient seemed dull mentally and the question of tuberculous meningitis was raised. However, the following day the patient was bright and promptly recovered. The spinal fluid on April 27 showed 23 cells and a faintly positive globulin reaction and on May 1 was normal. The child was discharged well the following day. When seen a month later, he seemed to have completely recovered without sequelæ. The diagnosis of an acute encephalitis following measles was made.

(2) *Infantile cerebral paralysis* (*hemiplegia et diplegia spastica infantilis* of Strümpell[101]) is characterized by a sudden onset with fever, convulsions and vomiting and followed by spastic hemiplegia or diplegia often with ocular paralysis and later by athetosis, epilepsy and mental retardation.

(3) The *encephalitic type of acute poliomyelitis* is similar to the disease described above. This type is so rarely observed even during epidemics of acute poliomyelitis (Wickmann,[102] Römer,[103] Peabody, Draper and Dochez[104]) that its relationship to acute poliomyelitis is still questionable.

Typical cases of the two types described above can be differentiated from epidemic encephalitis by the clinical course, the persistence of paralysis and the sequelæ.

(4) *Encephalitis from lead* is occasionally observed in infants.[105] Other evidences of lead poisoning, the history of ingestion of paint and the presence of frequent convulsions usually lead to a positive diagnosis.

Tuberculous Meningitis.—Great difficulty, especially in children, is found in the differentiation of epidemic encephalitis from tuberculous meningitis. Early in the course of either disease the clinical pictures may be so similar that not until several days have elapsed, or until characteristic changes have occurred in the spinal fluid, does the diagnosis become clear.

Case 43.—E. G. R., a white girl, aged 3½ years, was admitted to the Harriet Lane Home July 17, 1919. Her father had active tuberculosis and there had been intimate contact between the child and the father. The past history had been uneventful. The present illness began suddenly the night of July 13 with vomiting. On the following day the patient vomited again and for the next few days felt tired and weak. July 17, while on a street car, she had a convulsion which was generalized. Following this there were five or six general convulsions. The patient was admitted in a state of stupor with a temperature of 101° F. Physical examination revealed nothing at that time but an unconscious, convulsive child with hyperactive reflexes. The leucocyte count was 13,000 and the spinal fluid practically normal. The tuberculin test was positive. The following day irregular respiration of the Cheyne Stokes type was present and the convulsions continued. Strabismus and a facial weakness developed. The lumbar puncture was repeated on July 19 showing 23 cells per c. mm., a positive globulin reaction and film formation. Subsequent punctures revealed a steady increase in cells (see c. s. f. chart) but no tubercle bacilli could be found. (Guinea-pigs inoculated with the fluid showed no evidence of infection eight weeks later). However, in view of the exposure, positive tuberculin reaction, spinal fluid changes and comatose state with Cheyne Stokes breathing, tuberculous meningitis was considered the most probable diagnosis. The temperature rose on July 23 to 105° F.; following this it fell to normal and remained so. During this period the patient remained in a stuporous state and when aroused was very irritable. On July 27 she began to recognize her surroundings and became less drowsy but extremely irritable. She steadily improved and by the middle of August was walking about the ward apparently normal. She was discharged at this time and reports from the mother indicate complete recovery without sequelæ.

Case 44.—M. W., a colored girl, aged 2 years, was admitted to the Harriet Lane Home May 20, 1920, with the complaint "sleeps all the time." This condition began the middle of March with trembling of the hands, weakness and unsteadiness in walking. She became less playful, but otherwise nothing abnormal was noted until April 25, when she fell. After this her gait became unsteady, and the tremor of the hands was more noticeable. On May 13 she became very drowsy and following this remained in bed sleeping most of the time. No vomiting, convulsions, or paralyses were noted. Examination on admission showed a well-nourished colored girl lying in a state of deep lethargy from which it was impossible to arouse her. The extremities were somewhat spastic, but the reflexes were not exaggerated. The temperature was normal, the leucocyte count 10,000, the Pirquet and intracutaneous tuberculin reaction with 0.1 mgm. O. T. negative. Lumbar puncture showed a clear fluid, 41 cells per c. mm. and a positive globulin reaction. No films formed in the fluid. The Wassermann test was negative with the blood and spinal fluid. The X-ray picture of the chest showed normal lungs. The

eye-grounds were likewise normal. The diagnosis of epidemic encephalitis was made. On April 22 retraction of the head was noted; the first evidence of meningeal irritation that the patient had shown. This became more marked and on the following day there was opisthotonos. The temperature remained normal. Successive lumbar punctures showed an increase in cells up to 152 with a positive globulin reaction. The patient's condition grew rapidly worse and she died May 24. The question as to diagnosis between epidemic encephalitis and tuberculous meningitis was not settled until autopsy. The development of signs of meningeal irritation and the steady increase in the number of cells with an increasing amount of globulin spoke in favor of the latter, although the absence of fever, the negative tuberculin tests, X-ray examination and inability to demonstrate tubercle bacilli in the spinal fluid were against this diagnosis. Autopsy showed a typical tuberculous meningitis.

There are several points that aid one in this problem, particularly as far as children are concerned:

First. The onset in tuberculous meningitis is more gradual while in encephalitis it is often abrupt and acute, as a rule, resembling the onset of any acute infectious disease.

Second. The temperature in encephalitis is high at the onset, subsiding rather rapidly, whereas in tuberculous meningitis it is not high at onset, but rises toward the end.

Third. Clinical course.—Tuberculous meningitis in children is usually fatal in three weeks, whereas encephalitis may be prolonged over a period of months.

Fourth. Evidence of tuberculosis elsewhere (tuberculosis of the skin, tubercles of the choroid, X-ray of chest, tuberculin reaction).

Fifth. The cerebrospinal fluid.

(a) The pressure is usually normal or very slightly increased in encephalitis, whereas it is usually under increased pressure in tuberculous meningitis.

(b) The cell count in encephalitis may be high at onset, rapidly falling to normal. In tuberculous meningitis, it is not much increased at onset, but rises rapidly as the disease progresses.

(c) The amount of globulin is greater in tuberculous meningitis than in epidemic encephalitis.

(d) Films rarely form in encephalitis in typical manner as in tuberculous meningitis, probably because the amount of globulin and fibrin are less.

(e) Tubercle bacilli may be demonstrated in the films in tuberculous meningitis if repeatedly searched for. Guinea-pig inoculation may be positive.

Poliomyelitis.—In its typical form, poliomyelitis is easily distinguishable from epidemic encephalitis. The nature of the paralyses is entirely different, as are the clinical course and the sequelæ. The chief difficulty lies in the following types of cases:

1. Acute fulminant cases with high fever, great prostration, changes in the spinal fluid and death in a few hours.

Case 45.—D. G., a white girl, aged 6 years, was taken ill on June 18, 1919, with fever and drowsiness. Anorexia developed and she became more lethargic. On June 21 she became unconscious. The fever continued and she was brought to the hospital June 22. Examination on admission showed a somewhat poorly nourished white girl lying in an unconscious state with eyes half closed. The respirations were slow and irregular with periods of apnœa. There was drooling of saliva from the mouth. The deep reflexes were obtained and there was a positive Kernig's sign. The Babinski was positive on the right. The temperature was 101° F.; the leucocyte count was 18,000. Lumbar puncture showed a clear fluid with 55 mononuclear cells per c. mm. and a negative globulin reaction. On the following day the patient had repeated convulsions. The temperature rose rapidly reaching 108° F. She died June 24. No autopsy was performed. In such cases it is impossible to make a differentiation clinically. The presence of an epidemic, however, may be an aid.

2. *The Encephalitic Form of Poliomyelitis.*—This form was discussed briefly above and is extremely rare, even during epidemics of poliomyelitis. One must depend here too on the presence of an epidemic.

We may add that during the period of observation of these cases (1919-1920) poliomyelitis was extremely infrequent in Baltimore. There is no evidence clinically, epidemiologically, or histologically of the identity of the two diseases and one must assume, therefore, that they are distinct entities (see discussion by McNalty[8]).

3. In the form of encephalitis described above (peripheral or polyneuritic type) it may be impossible to differentiate from the same form of poliomyelitis. Symmetrical flaccid paralysis does occur in the latter disease, but it is rare. The occurrence of other cases of encephalitis at the same time, the complete recovery and the optic neuritis in one patient inclined us to regard these cases as encephalitis rather than poliomyelitis.

Syphilis of the central nervous system must always be excluded by the result of the Wassermann test with the blood and cerebrospinal fluid. It is possible for a patient with cerebrospinal syphilis to acquire epidemic encephalitis, but such an occurrence would be unusual. The pupillary changes and the transient pareses always make one think of syphilis. In general, however, the syphilitic diseases of the nervous system are more insidious in their onset and more chronic in their course, and changes in the optic discs are far more common.

Tumor of the Brain.—There is rarely difficulty in differential diagnosis between epidemic encephalitis and tumor of the brain. The former is usually a febrile disease characterized by transient and recurrent paralyses not referable to a single lesion. Moreover, choked disc, so common with intracranial neoplasms, has been rarely observed in instances of epidemic encephalitis. A rapidly growing infiltrating tumor of the pons or mid-brain must be excluded in every instance. A careful study of the sequence of the symptoms, together with repeated examination of the fundi and subarachnoid fluid throughout a period of observation, will usually prevent an error in diagnosis.

Abscess of the Brain.—We have met with one instance in which during life encephalitis was suspected, but at autopsy an abscess of the brain was discovered.

Case 46.—F. M., a colored boy, aged 3½ years, was brought to the Harriet Lane Home September 27, 1920, with the history that he had been well until four weeks ago and since that time had been listless and drowsy, had complained of pains in the abdomen and had vomited after every feeding. There had been no convulsions and the mother did not think there had been fever. Examination on admis-

sion showed a well-nourished colored boy lying in an apathetic state. However, he was quite conscious and apparently understood what was said to him. There were no signs of meningeal irritation, there was slight right facial weakness and the deep reflexes could not be obtained; otherwise the examination was negative. Ophthalmological examination showed a bilateral choked disc. The temperature was normal and remained so during the entire stay in the hospital. The leucocyte count was 14,000. The spinal fluid was under slightly increased pressure with 8 cells per c. mm. and a faintly positive globulin reaction. Epidemic encephalitis was considered, although the bilateral choked disc was evidence against this diagnosis. The patient became more drowsy, he vomited continually and went into coma on September 30. He died the following day. After his death the mother stated that two months before admission the child fell upon a plank, running a nail into his face. This wound healed promptly and the incident was forgotten. Autopsy showed an abscess of the left temporal lobe (staphylococcus aureus) with a punched out hole in the left temporal bone. There was no external evidence of this wound.

The clinical picture of abscess may be very similar to that of encephalitis with an acute onset, high fever, delirium or stupor, slight spinal fluid changes, coma and death. One's suspicions should be excited by the presence of a focus of infection (chronic otitis or mastoiditis or sinusitis) or of an injury or evidence of infection elsewhere in the body as well as by localizing symptoms. Blood cultures may be of value here.

Drug Poisoning.—Attention was called by Hassin and Wien [106] to the similarity of the state resulting from barbital (trional) poisoning to that of encephalitis. Such a condition may perhaps be produced by any hypnotic drug. The history and the absence of change in the spinal fluid should eliminate this source of error.

Typhoid and Other Acute Febrile Diseases.—At the time of onset it may be impossible to determine whether we are dealing with an acute infectious disease with mental and cerebral symptoms or with an encephalitis. This is particularly true in children who so frequently react to acute infections with delirium and psychotic states as well as stupor. The differentiation may be possible only after observing the course of the disease, or the results of the laboratory tests.

Uræmia.—Uræmic states may present a picture closely resembling that of encephalitis and this must be kept constantly in mind. We encountered this difficulty in the following case:

Case 47.—B. McC., a white girl, aged 4 years, was admitted to the Harriet Lane Home March 28, 1920. She had been well until March 7, at which time she had high fever, was drowsy and complained of headache and pain in the eyes. There was a skin eruption which disappeared in a few days. The child continued to be drowsy and vomited repeatedly. There was fever but no paralysis was noted. On March 15 there was a tremor of both hands and, on the following day, the mother noted a strabismus. That evening the child had a general convulsion which was repeated on the succeeding day whereupon she was admitted to the hospital. Physical examination on admission showed a poorly nourished white girl lying in bed in a stuporous condition from which she could be aroused with difficulty. The respirations were slow and deep and suggestive of hyperpnœa. Otherwise the physical examination was negative. Shortly after admission the patient had a general convulsion. The temperature was 101° F., the leucocyte count 18,000 and the bicarbonate content of the blood serum 14 volumes per cent (Van Slyke). Spinal puncture showed clear fluid under somewhat increased pressure, 2 cells per c. mm. and a faintly positive globulin reaction. The urine was clear, its specific gravity 1018, acid, albumin in large amount, sugar negative, acetone a trace, guaiac negative; microscopically it showed a few white cells but no casts. The Wassermann test was negative with the blood and spinal fluid. The patient was given sodium bicarbonate by gavage. She rapidly became comatose, however, and the convulsions were repeated. The temperature rose rapidly to 105.5° F. and the patient died March 29. Lumbar puncture made post mortem showed 52 mononuclear cells per c. mm. and a positive globulin reaction. The clinical diagnosis was epidemic encephalitis. The anatomical diagnosis was chronic, diffuse nephritis. The brain was entirely normal both in gross and on microscopical examination.

The urine, blood pressure, fundus changes and renal function tests should aid in eliminating this source of error.

Peripheral paralyses due to other causes (lead, arsenic, diphtheria, alcohol, beri-beri, etc.).

Before making a diagnosis of the polyneuritic form of this disease it is necessary to eliminate the other causes of peripheral paralysis by the history and appropriate tests.

Prognosis

The mortality varies within wide limits in the various reports. In Germany it has been 30 to 40 per cent (Economo, Dimitz [15]), in France 25 to 30 per cent (Netter,[3] Bernard [28]), in England 20 to 50 per cent (McNalty,[8] Howell [107]) and in America 10 to 40 per cent (Abrahamson,[29] Boyd,[44] Wegeforth and Ayer [108]).

In our series of 81 cases there were six deaths, a mortality rate of 7.4 per cent. In general, if the patient survives the acute stage of the disease, the prognosis as to life is fairly good; as to complete recovery, however, the prognosis must be reserved owing to the frequency of sequelæ. Relapses are rare, but are said to have a high mortality (Netter).

Treatment

1. *General Measures.*—It is important, in the treatment of this disease, to bear in mind that epidemic encephalitis is not an acute disease of short duration, but may last over a period of many months and that convalescence is as a rule slow and tedious because of the frequency of psychic and motor disturbances as described above. Therefore, these patients should be carefully watched and protected over a sufficient period of time following the subsidence of the acute symptoms of the disease to make sure that recovery is complete before permitting them to take up their regular occupations. This becomes extremely difficult when we find that sequelæ may arise months after apparent recovery.

At the onset of symptoms the patient should be placed in a separate room and kept as quiet as possible. Careful supervision is required because of the development of excitation reactions (delirium, mania, chorea, etc.). General isolation of the patient is advisable, but it is not necessary to isolate so strictly as with diseases of more infectious a nature. We know of no disease in which careful nursing plays a more important rôle, for these patients are generally quite helpless.

The diet should be liquid or soft since difficulty in swallowing is often present and gavage may be necessary. One of our patients in deep lethargy had to be fed by gavage for five weeks. Sedatives may be necessary during the state of agitation, delirium and motor excitation. During convalescence, also, the patient should be protected in every way possible and fatigue and excitement avoided. It is a mistake to regard the patients as "cured" after the acute symptoms have subsided, for during convalescence the unpleasant sequelæ, insomnia, psychic disturbances, change in disposition and general motor overactivity, often make their appearance. The parents and relatives should be forewarned of the possibility of the appearance of these symptoms, and parents should be advised not to punish children for odd and untidy acts entirely beyond their control. The insomnia is most difficult to combat. We have had no success with hypnotics, baths or packs, and have allowed the patients to sleep during the daytime, receiving their food when awake. Most of them have improved with time, but great patience on the part of parents and relatives is required. Leahy and Sands advise that these patients be kept awake during the daytime and that occupational therapy be employed.

2. *Drugs.*—There is no specific for this malady, although a variety of drugs have been used. Netter,[109] basing his opinion on the work of Crowe and Cushing, believes that hexamethylenamine is of value when given by mouth. If the urine be carefully watched there is no harm in giving this drug, although its value is problematical. The same author also advises pilocarpin in patients with sialorrhea, believing that in this way one may influence the elimination of the virus. Although sialorrhea was frequently observed in our cases, we have had no experience with the use of sialogogues.

Salvarsan has found favor with various observers (Netter,[109] Fourrier [110]), the latter finding marked improvement following its administration. The specificity of arsenic in these cases is doubtful. Sedatives, as mentioned above, are helpful in the acute stage, although their use in the chronic sleeplessness of convalescence is disappointing.

3. *Intraspinous Therapy.*—Repeated withdrawal of spinal fluid during the acute stage of the disease, especially if the fluid be under increases pressure, is generally regarded as of value. Reasoning by analogy with poliomyelitis Netter,[109] Sabrazes and Massias,[111] Marinesco,[8] Sicard [112] and others advise the intraspinous injection of the blood of convalescent patients, and Cantieri and Vegni [113] the intraspinous injection of the spinal fluid of convalescent patients. The scarcity of available convalescents makes this form of therapy limited, unless one encounters a large local epidemic. It is quite possible that the local irritation produced is the factor of value in this treatment as is probably the case in the improvement after the injection of other sera intraspinously (antitetanic serum Laubie,[114] grippe serum Fendel,[115] etc.).

4. *Miscellaneous.*—Netter [23] has been an exponent of the use of the "abscess of fixation," that is, the production of a sterile abscess by injection of turpentine in a remote part of the body. He reports marked improvement following its use. The injection of various sera (grippe serum Fendel,[115] Oehming,[39] auto-serum Brill,[116] Bourges and Marcandier [117]), the intravenous injection of hypertonic salt solutions (Hoffman [118]) and the injections of colloidal iodin solutions (Claisse [119]) have been recommended. We have had no experience with these measures. We may say, in summary, that there is no specific therapy for this disease. Each case must be treated on its own merits with quiet, rest, hypnotics, careful nursing and feeding and spinal punctures during the acute stage, and careful attention during convalescence. Individual symptoms should be treated symptomatically as they arise.

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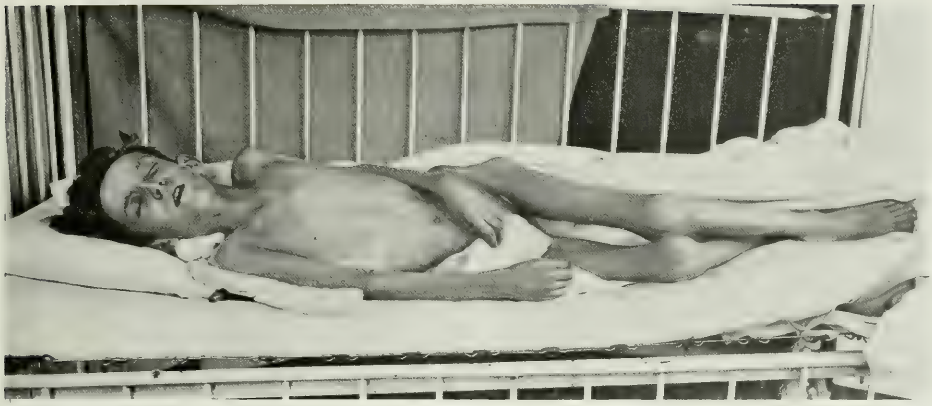

FIG. 1.—(Case 2.) A patient with marked lethargy.

FIG. 2.—(Case 2.) Same patient as in Fig. 1, two months later, after disappearance of lethargy. The mask-like facies was still present.

FIG. 3.—Same patient as Figs. 1 and 2, five months later. The face was still mask-like.

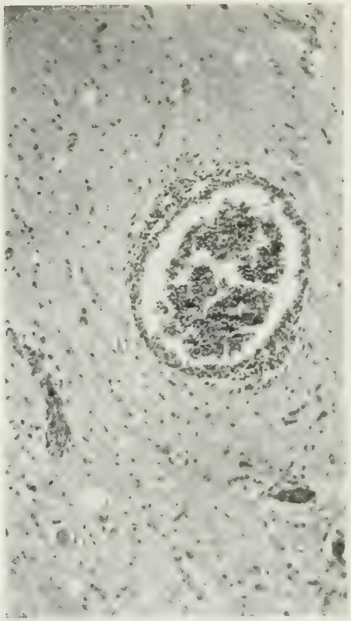

FIG. 4.—(Case 6.) × 146. Marked perivascular round-cell infiltration. Area was in the substantia nigra.

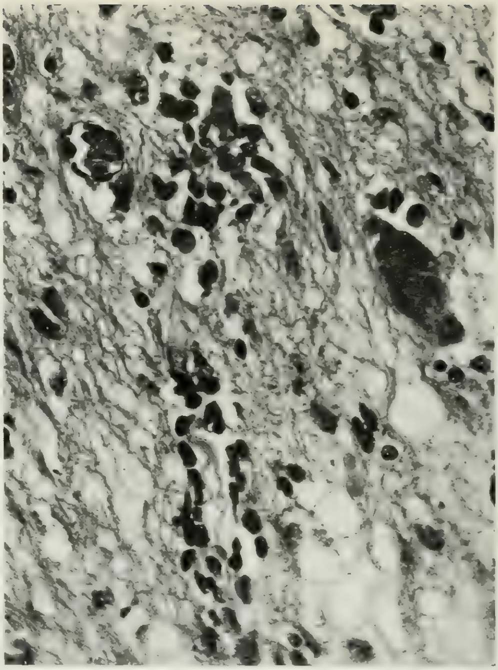

FIG. 5.—(Case 6.) × 480. Hight power of area of substantia nigra to show a collection of round cells near a small blood-vessel.

FIG. 6.—(Case 17.) Bilateral ptosis and mask-like facies in an infant of 7 weeks.

FIG. 7.—(Case 19.) Unilateral ptosis—"ironed-out" face.

Fig. 8.—(Case 29.) Facial expression after subsidence of choreiform movements.

Fig. 9.—(Case 34.) Left facial palsy in epidemic encephalitis.

Fig. 10.—(Case 35.) Expressionless face.

Fig. 11.—(Case 35.) Same patient as in Fig. 10, three months later.

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STUDIES ON EXPERIMENTAL RICKETS

III. A PATHOLOGICAL CONDITION BEARING FUNDAMENTAL RESEMBLANCES TO RICKETS OF THE HUMAN BEING RESULTING FROM DIETS LOW IN PHOSPHORUS AND FAT-SOLUBLE A: THE PHOSPHATE ION IN ITS PREVENTION

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As is now well known, Mellanby[1] has reported the production of rickets in puppies by diets deficient in their content of fat-soluble A,* or an antirachitic factor having a similar distribution. Unfortunately, it has not been possible to evaluate Mellanby's results by reading his published writings, since he has never supplied objective proof of the production of rickets in the form of illustrations showing the appearance of his specimens through the microscope. This omission has been unfortunate, inasmuch as the diagnosis of this disease can be made with certainty only on the evidence derived from microscopic changes in the bones. The gross deformities which characterize rickets can be simulated in other pathological conditions, as, for example, the osteoporosis which develops in growing puppies during confinement in small cages. Evidence afforded by the X-ray is not completely convincing. X-ray examination fails to disclose the exact nature of the pathological process in operation, and to differentiate rickets, as it commonly occurs in children, from related conditions such as can be produced in animals through dietetic influence.

In a previous publication[2] we described 11 diets, some of which produced gross deformities resembling those seen in rickets. The nature of the faults of these diets was in all cases clearly pointed out. These diets included formulæ for food mixtures in which there was a deficiency of but one dietary factor, *i. e.*, calcium or fat-soluble A. The other diets described were faulty in the following respects: lack of fat-soluble A and excess of calcium, and low calcium and low fat-soluble A. Since the preparation of the earlier paper we have had occasion to observe also the effect of changing the plane of intake of the phosphate ion on the growing bone.

* We are using provisionally the term fat-soluble A, to designate the organic factor which may play a special rôle in bone development. In a later paper we shall present further experimental data, of a nature which will serve better than those contained in the present discussion for deciding whether the organic factor contained in cod-liver oil, to which that substance owes its therapeutic value in certain diseases affecting the skeleton, is identical with the factor which we here refer to as fat-soluble A.

Experiments Designed to Show the Effects on the Skeleton of Diets Low in Their Content of Fat-Soluble A and Phosphorus

In order to bring out the effects of varying the plane of the phosphate ion in the diet on bone development, we have employed the following food mixtures:

Diet of Lot 2667

Rolled oats	40.0
Flaxseed meal	8.3
Sodium chloride	1.0
Calcium carbonate	1.5
Dextrin	49.2

100 gms. of this mixture contained 0.6714 gms. of calcium and 0.2165 gms. of phosphorus.

This diet is deficient to some extent in its protein content. The protein, both in amount and quality, is adequate, however, to induce growth, though at a rate distinctly below the

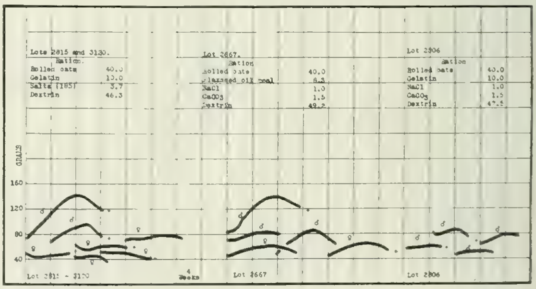

Chart I.

The Curves in this Chart Illustrate the Weight Changes in Young Rats Fed the Diets Described in the Text.

maximum. But no development is possible on this food mixture unless it is supplemented by some substance which will serve as a source of the factor, fat-soluble A. This diet, as stated, is deficient in fat-soluble A, and also contains a relatively small amount of phosphorus.

When young rats weighing from 20 to 70 gms. between 18 and 50 days old are confined to this diet, without the addition

of any substance supplying fat-soluble A, they soon cease to increase in weight and then rapidly decline (Chart I). After an interval which varies markedly, depending principally on the age of the animal but to some extent on individual resistance, they all develop xerophthalmia. In our extensive experience no animal has ever recovered from this condition unless a substance capable of acting as a source of fat-soluble A was added to its diet. On the addition of such a substance, *e. g.*, butter fat or cod-liver oil, animals suffering from xerophthalmia steadily improve and return to normal health. While recovery is taking place, growth is resumed. With this diet we have conducted scores of experiments; the general behavior of the animals placed upon it and the development of xerophthalmia have occurred with unvarying regularity as described.

DIET OF LOT 2806

Rolled oats	40.0
Gelatin	10.0
Sodium chloride	1.0
Calcium carbonate	1.5
Dextrin	47.5

100 gms. of this mixture contained 0.6414 gms. of calcium and 0.1580 gms. of phosphorus.

This diet contains protein of good quality and in sufficient amount to enable young rats to grow at a fairly rapid rate, provided the faults in the diet are corrected by suitable additions. The principal defect in this diet is a deficiency in fat-soluble A. Without the addition of this factor no growth is possible, and severe xerophthalmia develops followed by death. A second defect in the diet sufficiently grave to interfere in a measure with growth is the lack of sufficient phosphate ion. While growth can take place on the formula tabulated above, supplemented with some substance acting as a source of fat-soluble A, it is not so satisfactory as when the sodium chloride and calcium carbonate are replaced by a salt mixture containing not only these substances but suitable amounts of the phosphate ion as well.

Methods.—All the animals in the experiments were killed with ether. After careful postmortem examination various bones and some of the soft tissues were saved for histological examination.

In preparing bones for microscopical examination we have usually used the method recommended by Pommer—decalcification in Müller's fluid for periods ranging from two to fourteen days after preliminary fixation in formalin. The bones were dehydrated, embedded in celloidin and the blocks cut into sections from 10 to 60 microns thick. While we have used thionin and other dyes and have treated a large number of sections with silver nitrate after the method of Von Kossa, no staining technique has proved to be so satisfactory as that in which a combination of Ehrlich's hæmatoxylin with water soluble eosin is used. We have controlled the material which was partially decalcified in Müller's fluid by means of celloidin and frozen sections of undecalcified bones.

The Pathological Condition Produced in the Skeleton by the Administration of Diets Deficient in their Content of Fat-Soluble A and Phosphorus.—The diets described above (diets of lots 2667 and 2806) give rise to pathological pictures in the bone presenting a wide range of variation. At one extreme, the picture closely resembles that of rickets as that disease manifests itself in the human being, at the other extreme, osteoporosis.

The autopsy findings in the rats whose condition most closely resembled rickets, were as follows: The animals were undernourished and under-developed. Their coats were rough and uneven. Xerophthalmia was invariably present. The teeth were loose; the incisor teeth were easily fractured or extracted. In some animals spontaneous fracture of one of the incisors had occurred. The cranial bones were soft. The costochondral junctions were enlarged and the oval swellings formed were unusually long. In some rats there was moderate displacement inwards of the middle tier of costochondral junctions. In some animals a few fractures of the ribs marked by callous formation but without deformity of the costal arch were present. The spleen was atrophic in some animals, in others more or less enlarged. The thymus was atrophic. There was little or no fat. The long bones were straight and were not factured. The ends of the long bones were greatly enlarged. The femur and tibia were easily cut and gave little evidence of the presence of calcium salts until the knife had entered well into the shaft. Gross examination of the cut surface revealed a broad zone of a pale tissue, 2-4 mm. in width, lying between the epiphyseal cartilage and the shaft (the rachitic metaphysis).

The epiphyseal cartilage in some animals was increased in width throughout the entire area of the section. It was more common, however, to find it of a normal width or a width not greatly exceeding the normal except at the periphery of the bone. At the periphery prolongations of cartilage extended diaphysealwards well into the metaphysis. Higher magnification showed the matrix of the cartilage to be unusually abundant in proportion to the numbers of cells present. The undifferentiated portion of epiphyseal cartilage was represented by a thin zone in which the cells appeared to be unusually far apart. The proliferative zone contained columns of cells aggregated in bullet-shaped masses, the points of which were directed toward the undifferentiated cartilage. Each bullet-shaped mass was separated from its fellows by a broad band of matrix (Fig. 3). As the metaphysis was approached, the cells of the proliferative zone, which were small and often flattened, with lense-shaped nuclei, grew larger and assumed a nearly spherical form, and at the same time underwent a progressive loss of affinity for the basic dyes. The nuclei became larger and the staining reaction approached more nearly that of the osteoid tissue in the metaphysis (Fig. 4). At the points of junction of the cartilage with the metaphysis the cartilage cells lost their columnar arrangement and became heaped together in disorderly masses. There was no evidence of calcium deposition in the zone of proliferative cartilage corresponding to the zone of provisional calcification in the normal bone, or else the calcification was fragmentary. The cartilage was invaded to a considerable extent by blood vessels from the marrow cavity, but did not appear to be greatly disorganized. The invading vessels for the most part were slender and appeared to advance, in some instances at least, along the strands of matrix between the columns of cells.

The metaphysis was composed chiefly of osteoid trabeculæ but also of blood vessels carrying with them marrow elements, tongue-like bands of cartilage cells of various thicknesses continuous with the epiphyseal cartilage and of islands of cartilage cells. The trabeculæ were broad. Many of them contained central cores of uncalcified cartilage and in almost all of them single cartilage cells could be identified or small groups in various stages of transition to a state indistinguishable from the cells of the osteoid itself. The trabeculæ were separated from each other by wide blood channels each of which was surrounded by a small amount of delicate connective tissue and a few marrow elements. The osteoblasts covering the trabeculæ were flattened, spread out like endothelial cells and had small nuclei. The osteoid of the trabeculæ was for the most part laid down in the form of lamellæ, but the lamellar arrangement could not always be made out. The bone corpuscles varied in size, but by far the majority were, to judge from their nuclei, small cells. The large prolongations of the epiphyseal cartilage into the metaphysis seemed to retain for the greater part of their extent the normal staining reaction to hæmatoxylin (Fig. 4). The smaller prolongations, those consisting of thin strands of cartilage cells, and the

terminal portions of the large prolongations just alluded to, seemed to lose their staining reaction almost entirely. The loss of the staining reaction appeared to occur in the neighborhood of the blood vessels and apparently depended on the intimacy of contact between the cartilage cells and the vascular elements. It was most marked in the strands or groups of cartilage cells which had become incorporated in the interior of the osteoid trabeculæ. As the cartilage cells came into close relationship with the marrow elements, they also appeared to undergo changes in their morphology. The cell bodies became smaller and took on a vesicular appearance. Around the cell body there developed a homogeneous material having the same staining reactions and outward appearance as the uncalcified material formed by the osteoblasts. In the osteoid trabeculæ removed some distance from the epiphyseal cartilage, where the change taking place in the cartilage cells appeared to be far advanced, the cartilage cells were so reduced in size that they could be distinguished from the osteoblasts only by their oval cell capsule and the vesicular appearance of the cytoplasm, or could be differentiated with the greatest difficulty or not at all. In some osteoid trabeculæ cartilage cells could be found in all stages of transition to osteoblasts.* The cartilage cells embedded in the osteoid trabeculæ seemed to be stimulated to reproductive activity, for areas were seen where single cells had obviously given rise to from two to five daughter cells (Fig. 5). Cell division was apparently accomplished by amitosis. The cartilage cells composing the small islands in the metaphysis underwent the changes just enumerated. The cartilage cells composing the larger islands, however, retained their ordinary staining reactions except at the periphery where an abrupt change to osteoid took place. These large islands of cartilage cells appeared to be insulated by thin rims of osteoid. It is important to note that the tendency toward calcium deposition must have been very marked even in those animals that exhibited the pronounced changes of a rachitic nature just enumerated, for in the bones of the great majority of them linear deposits of lime salts were found which passed transversely across the metaphysis at right angles to the long axis of the bone. These deposits were sharply separated from the shaft of the bone, on the one hand, and from the proliferative zone of cartilage on the other. In some animals a single bone showed two or three of these evidences of abortive attempts at healing (Figs. 8 and 9). Such bones were striking in their resemblance to the bones of children showing the lesions of so-called "healing rickets."

In the shaft of the bones of the animals showing extreme changes of the nature just described, the formation of osteoid tissue about the trabeculæ was markedly increased (Fig. 6). The small trabeculæ of calcified bone were completely surrounded by broad areas of osteoid and the periosteum was widely separated from the cortex by masses of osteoid tissue (Fig. 7). New bone formation in fibrous tissue was found occasionally beneath the periosteum in the region of the metaphysis. The osteoid tissue on the periosteal side of the cortex was traversed by numerous blood vessels. Here and there it was possible to find evidences of bone resorption. The evidences of resorptive activity in the metaphysis were very slight.

In animals at the other extreme the abnormality was of the nature of an osteoporosis. The epiphyseal cartilages were perhaps slightly irregular, but they were narrow. There was no evidence of abnormal persistence of the cartilage cells. Heavy continuous deposits of lime salts were present in the diaphyseal border of the cartilage, and this latter terminated abruptly in contact with the shaft. The marrow cavity was crowded with hæmatopoietic cells. The cortex was thin but completely calcified. In the medullary cavities of some bones a few very thin, completely calcified trabeculæ were present, but in others the only bone to be found was the cortex of the shaft. In some of the rats showing these extreme osteoporotic changes the trabeculæ were invested with fibrous tissue and around them were to be found basophile cells with single nuclei and large granules. The evidences of resorptive activity in the trabeculæ were well marked. No osteoid was present anywhere. The gross *autopsy findings* in these animals were in accord with the picture which the microscope revealed in the sections. There were no deformities of the skeleton of any sort, but the bones were abnormally slender, a finding especially noticeable in the ribs, because of the contrast between the thin shaft and the costal cartilages. The latter appeared to be of normal size or even larger than normal. The bones gave evidence of the presence of calcium salts when an attempt was made to cut them; they were brittle and fragile, although there were no fractures to be found.

All stages of transition were found between the two extremes of the pathological process induced in the skeleton by the diets low in fat-soluble A and phosphorus, and by far the greater number of the animals examined may be said to have exhibited pathological changes of a mixed nature. In all these animals of the intermediary group the pathological picture presented had a rachitic background. The deposition of the calcium salts in the cartilages was irregular, and areas were found in the proliferative zone in which lime salt deposits were not present. The cartilages in most of the rats were extremely irregular, and in a considerable number of animals there was persistence of the cartilage with the formation of a thick proliferative zone (Fig. 10). In this thick proliferative zone large defects were noted due to invasion of the cartilage by blood vessels. In some of the animals, although calcification of the cartilage had occurred, prolongation of the cartilage was pronounced. Other animals showed cartilage cells in calcified as well as uncalcified portions undergoing the changes already alluded to which have been probably wrongly regarded as signs of degeneration. In almost all the rats showing these mixed lesions there was a narrow metaphysis or a partial metaphysis, *i. e.*, one extending part way across the bone. All the changes characteristic of rickets in the human being were present with the exception of the absence of calcium deposition in the proliferative cartilage. The number of trabeculæ in the shaft, however, was greatly reduced and, while even broad zones of osteoid were in evidence about the trabeculæ, there were many signs that the trabeculæ were in process of resorption and were being removed from the medullary cavity (Fig. 11). The gross changes in the skeleton consisted merely in varying degrees of enlargement of the costochondral junctions and the ends of the long bones of the extremities.

Experiments Designed to Show the Effects on the Skeleton of Diets Low in Their Content of Fat-Soluble A, But Having an Adequate Amount of Phosphorus

Diets of Lots 3120 and 2815

Rolled oats	40.0
Gelatin	10.0
Salts (185)*	3.7
Dextrin	46.3

100 grams of this mixture contained 0.3150 gms. of calcium, and 0.5383 gms. of phosphorus.

* The disappearance of the staining reaction of the cartilage cells and the morphological changes enumerated do not indicate a degenerative process. On the contrary, the existence of the cartilage cells appeared to be prolonged in a remarkable manner. As the cartilage was invaded by the capillaries, its cells became embedded in osteoid through the activity of the osteoblasts. In this envelopment of osteoid they persisted and became indistinguishable from the cells of the osteoid investment. Our experience would lead us to believe that the cartilage cells may undergo metaplasia into the cells of the osteoid.

* Composition of salt mixture 185.

NaCl	0.173
$MgSO_4$ (anhydr)	0.266
$NaH_2PO_4 + H_2O$	0.347
K_2HPO_4	0.954
$CaH_4(PO_4)_2 + H_2O$	0.540
Fe citrate	0.118
Ca lactate	1.300

Of this mixture 3.7 gms. were added to every 100 gms. of ration.

This diet, as is easily seen, contains protein of fairly satisfactory amount and quality. The properties of the mixture differ from those of the diets of Lots 2806 and 2667 in that there is contained a salt mixture having among other things the sodium, chlorine, calcium and phosphate ions in satisfactory amounts. The diets of Lots 2806 and 2667, however, lacked the phosphate ion from among the necessary supplements for the inorganic moiety of this diet. When young rats are restricted to this diet they soon fail to increase in weight and then slowly decline. They invariably develop xerophthalmia and die. This diet supplemented with some substance acting as a source of fat-soluble A induces growth and apparently fairly satisfactory nutrition over a considerable period.

The Pathological Conditions Produced in the Skeleton by Administration of the Diet Deficient in its Content of Fat-Soluble A, but Containing an Adequate Amount of Phosphorus

The eleven rats fed on this diet were all small and exceedingly mal-nourished. The eyes showed the characteristic xerophthalmia. The incisor teeth were fragile, loose in their sockets, and in some animals fractured. On removal of the skin there was a notable absence of fat. The thorax externally showed no deformity. On opening the thorax the arch of the ribs and cartilages was normal and there was no deformity or enlargement of the costo-chondral junctions. The shafts of the ribs appeared extremely thin, and the costal cartilages large as compared with them. No fractures of the ribs were present. The vertebral column was not bent. Both thymus and spleen were atrophic. The fore and hind legs were slender, but presented no deformities, fractures, or enlargements of the ends of the long bones. On section of the femur and tibia the resistance was greatly diminished, but the bones grated under the knife as the latter entered the shaft. On examination of the cut surface the cortex was found to be exceedingly thin. The marrow cavity extended close to or actually to the cartilage. There was either no sub-chondral zone of spongiosa, or an exceedingly thin one. The epiphyseal cartilage separating the large nucleus of ossification from the shaft appeared as a narrow band, the width of which was everywhere equal. By means of the binocular microscope there could be detected a continuous thin line of calcium deposit along the diaphyseal border of the cartilage.

On microscopic examination the results of gross examination were corroborated. The band of epiphyseal cartilage was found to be exceedingly narrow, and the columns of cartilage cells correspondingly short. Calcification of the proliferative zone lying in contact with the marrow cavity was complete. The character of the calcification corresponded closely to that found in the bones of animals whose growth is approaching completion, that is, each cell was completely enveloped by calcium deposit. The diaphyseal portion of the bone also had in some respects the appearance which characterizes the bones of animals whose growth has almost ceased, that is, there were few trabeculæ immediately adjacent to the cartilage; in some of the animals there were none (Fig. 12). When trabeculæ were present they were most numerous at the periphery, filling in the angles between the epiphyseal cartilage and the cortex. In the central portion of the bone in all the rats the marrow cavity was in immediate contact with the epiphyseal cartilage. The trabeculæ themselves were thin. In most of the rats they showed no osteoid borders anywhere. In some animals, however, the trabeculæ here and there were partially covered with borders of osteoid, some of which slightly exceeded the normal in thickness. The cortex was thin, completely calcified, and solid, *i. e.*, it did not contain spongy tissue. In the bones of the majority of the animals the signs of resorption were well marked. The trabeculæ were invested with layers of fibrous tissue, and in the periphery of the trabeculæ were countless numbers of rather large mononuclear cells conspicuous for the presence of basophilic granulations. These cells were thought to be connected with the resorptive process. In many trabeculæ holes and cavities could be found in which osteoblasts could be identified lying free, and in other places osteoblasts could be seen lying half in the calcified part of the trabeculæ and half in the osteoid border. Where osteoid borders were present, they appeared in the preparations stained with hæmotoxylin-eosin to be exceedingly pale, almost translucent, and the cell bodies of the osteoblasts in them, apparently strongly basophilic, were clearly defined in contrast. The osteoblasts themselves were large and in the trabeculæ appeared unusually close together. The nuclei were large and in those cells lying in the osteoid (where the cell bodies could be studied) were eccentrically placed in the cell body. The cell body was not round but irregularly oval. Near the nucleus at about the center of the cell was a large, round area of vacuolization about the size of the nucleus. It was by means of this area of vacuolization that the osteoblasts lying free in the holes in the trabeculæ or along the sides could be identified with such certainty. Many of the trabeculæ were covered with large numbers of osteoblasts. We are inclined to believe that the osteoid borders were caused not by growth but by removal of the lime salts, *i. e.*, halisteresis. The marrow, with the exceptions already noted, was normal. The basophilic cells which surrounded the trabeculæ were in no instance found in the marrow cavity.

Discussion

The first two diets used in these experiments (diets of lots 2667 and 2806) characterized by a deficiency in fat-soluble A and phosphorus, produced, as anticipated, xerophthalmia and changes in the skeleton. The xerophthalmia, as nearly as we could judge from gross inspection of the eyes, did not vary except in degree. The changes in the skeleton, however, exhibited a wide range of variation. In the animals at one extreme (in 13 rats) the ends of the long bones were characteristically large. The zone of proliferative cartilage in places was greatly broadened and continued in long processes toward the shaft. The cartilage cells in these processes and those elsewhere in contact with the marrow elements of the metaphysis exhibited the characteristic morphological changes and loss of staining properties so commonly seen in the rickets of human beings. The metaphysis itself was broad and composed, in addition to the vascular elements, almost entirely of osteoid trabeculæ. Calcification of the zone of the proliferative cartilage, corresponding to the provisional zone in the normal bone, was either entirely absent or very deficient. In almost all instances, however, there were broken linear deposits of calcium in the metaphysis, extending across it at right angles to the long axis of the bone. The pathological condition in these animals, showing the more extreme changes, exhibited all the fundamental characteristics of rickets, as it manifests itself in human beings, and may be said to have

borne a strong resemblance to a certain form of rickets occurring in the human being, characterized pathologically by linear zones of lime salt deposition in the metaphysis, indicating alternate healing and exacerbation.

In the animals at the other extreme (in 9 rats) the pathological condition corresponded to what is ordinarily included under the term osteoporosis. The epiphyseal cartilage was reduced to a narrow band. The transition between cartilage and shaft was abrupt. Calcification of the cartilage was regular and complete. The few thin trabeculæ were free from osteoid, and resorptive phenomena were abundantly in evidence. The pathological condition of the bones exhibited by the rats at this extreme of the series showed no evidences of rickets whatsoever, but on the contrary complete calcification of all elements both cartilage and trabeculæ.

The majority of the animals on these two faulty diets (27 rats), however, showed pathological conditions in the skeleton intermediary between the two extremes just briefly outlined. The trabeculæ were bordered with osteoid, not the thin border present in growing young animals, but broad zones comparable to those seen in the advanced rickets of children or even exceeding them in thickness. In all there was irregularity and broadening of the epiphyseal cartilage and defects in its calcification, in some instances most extensive, in others small. Some animals showed short metaphyses or partial metaphyses, in which were columns or masses of cartilage in an uncalcified or in an incompletely calcified state, displaying the morphological changes and alterations in staining reactions previously enumerated. The number of the trabeculæ were few, and in the trabeculæ, in particular those close to the cartilage, there were present abundant evidences of resorptive activity. The pathological conditions displayed in the bones of the rats showing these intermediary changes bore a marked resemblance to the not very advanced rachitis of human beings, when the healing process has been well established or has progressed far toward completion. An anomalous condition was present characterized by abundant calcium deposits in the cartilage on the one hand, and borders of the trabeculæ still uncalcified on the other, a well recognized and characteristic intermediary stage in the healing of the rachitis in human beings.

It is not possible to explain why all the rats fed the diets deficient in fat-soluble A and phosphorus failed to develop exactly the same changes in the bone. It is possible that the diets in question were not absolutely constant in their composition, not as regards a deficiency in fat-soluble A—for all the rats without exception developed xerophthalmia—but as regards some other substance. The stock supplies of flaxseed meal and rolled oats were replenished during the course of the experiments several times, and may have been derived from different sources.* A reason for suspecting that variations in the composition of the diets may have had some influence is that the animals which most consistently developed the marked rickets-like changes were the first animals of the series (those of the experiments completed before May 19, 1920). Another explanation, however, must be considered. As already pointed out, there were noted in all the animals, even in those showing the most marked rachitic-like changes, evidences of a strong tendency toward healing, manifesting itself in the deposition of calcium salts in the cartilage. In a large proportion of the animals the healing process, as measured by the extent of calcium deposition in the cartilage, appeared to be far advanced. If the composition of the diets was essentially constant, it is necessary to suppose that the abnormal conditions in the body induced by them, under which lime salts could *not* be taken up by the cartilage and the bone, were not very far removed from other conditions more nearly approaching the normal under which lime salts *could* be taken up by the cartilage and the bone, and that the former state could be transformed into the latter by slight changes in the animals' metabolism. In other words, the particular abnormal equilibrium of the forces concerned with calcification and ossification produced by the faulty diets was unstable. With the rapid decline in the nutrition of the animals occurring during the latter part of the experiments and the corresponding loss in weight, a complete cessation, or at least a great retardation in the rate of growth of the skeleton must have occurred. There must have come about, therefore, a diminished requirement for those substances essential to ossification and calcification, which were insufficiently supplied in the diets. Moreover, it seems probable that a supply of those substances may have been liberated from the tissues of the animals themselves, for example, as the result of the resorptive processes in operation in the bones. From a theoretical standpoint, therefore, it seems possible to think that the processes in operation in the animal leading to the development of the state of extreme malnutrition may have been instrumental in restoring to the organism conditions under which calcium deposition in the skeleton again became possible. For the development of rickets growth is necessary. If the deficiencies in the diet are of such a nature as to render growth of the skeleton impossible, rickets cannot develop. If growth were brought to an end in an animal already rendered rachitic through the administration of faulty diets, there is reason to expect that the rachitic lesion would disappear and a condition of osteoporosis develop. Whatever may have been the cause of the development of pathological changes in the skeleton, from the morphological standpoint, apparently so far removed from each other, there can be no doubt that the osteoporosis exhibited by some of the animals of the series was closely related to the healing process in evidence in the others, and represented merely the healing process in its completed state.

The pathological condition produced in the rat by the diet low in fat-soluble A, but containing a complete salt mixture (the diet of Lots 3120 and 2185) were absolutely constant and offered no difficulties of interpretation. In the entire series of eleven animals on this diet the band of proliferative cartilage was narrow, the junction between cartilage and shaft abrupt

* The content of the diets in calcium and phosphorus was calculated from the average composition of rolled oats and flaxseed meal, as compiled by Forbes, Sherman and others, and from the composition of the salt mixtures employed.

Fig. 1.

Fig. 2.

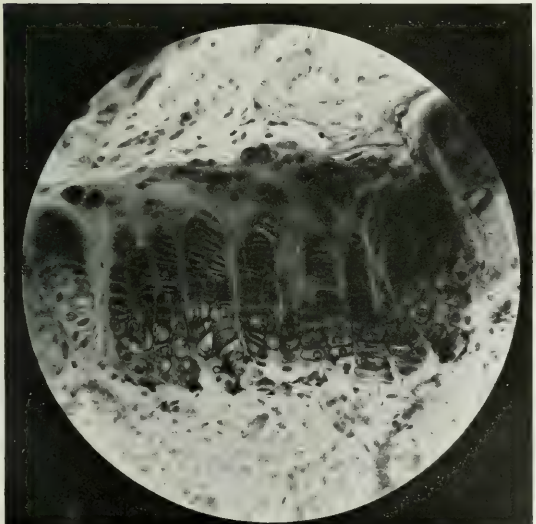

Fig. 3.

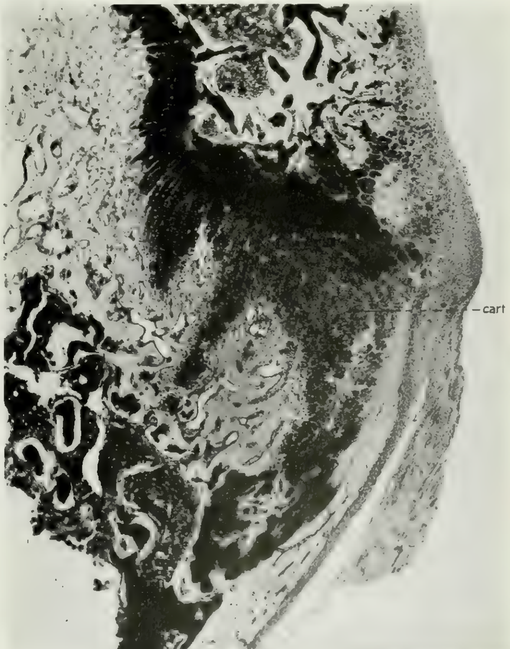

Fig. 4.

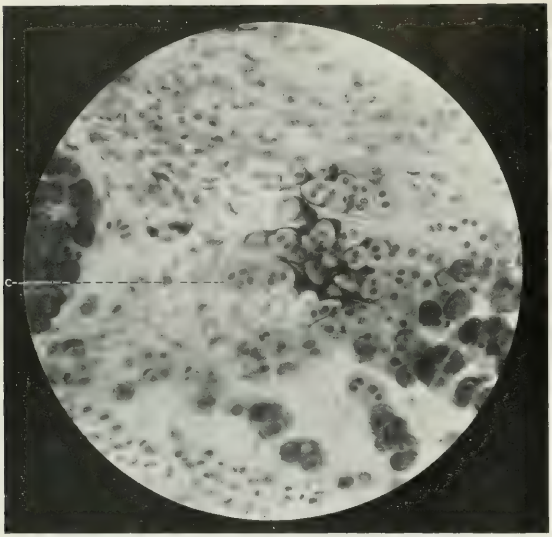

Fig. 5.

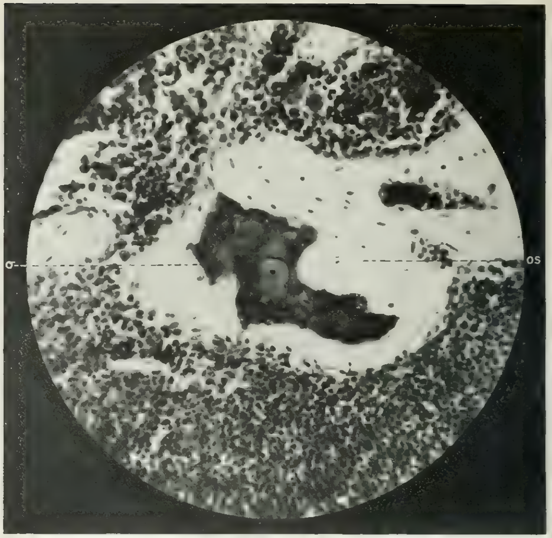

Fig. 6.

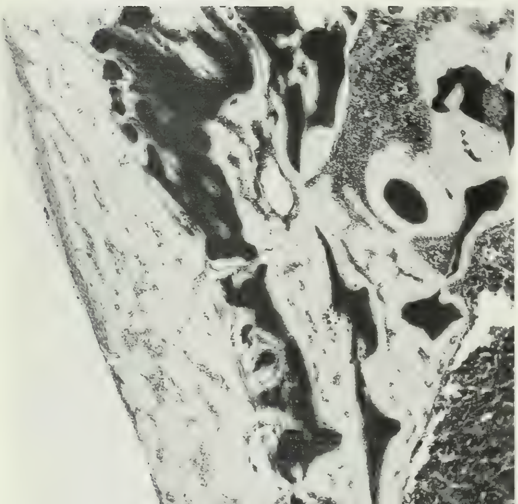

Fig. 7.

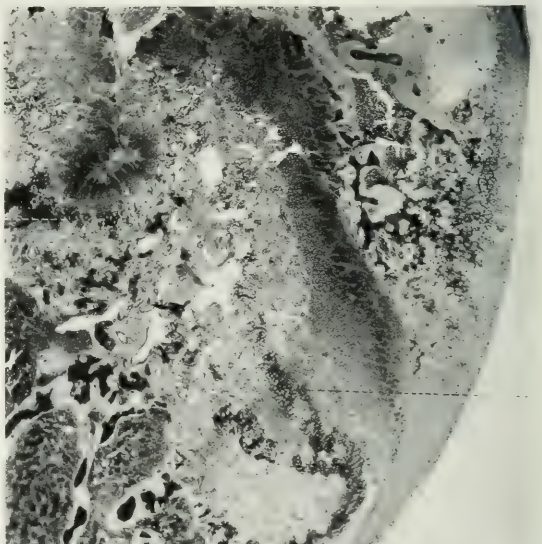

Fig. 8.

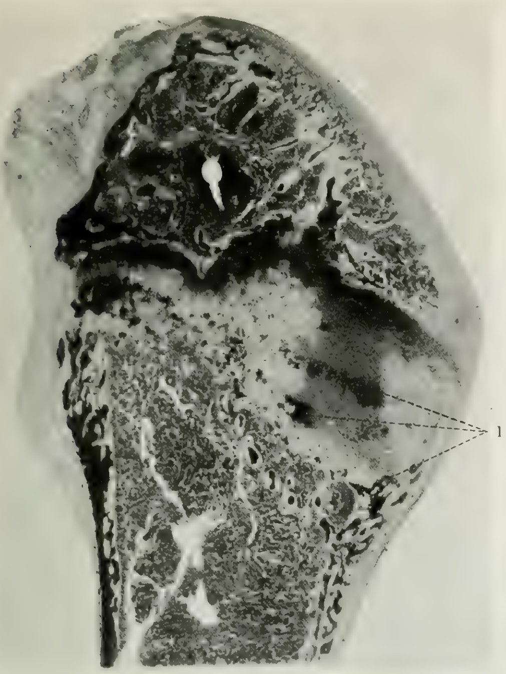

Fig. 9.

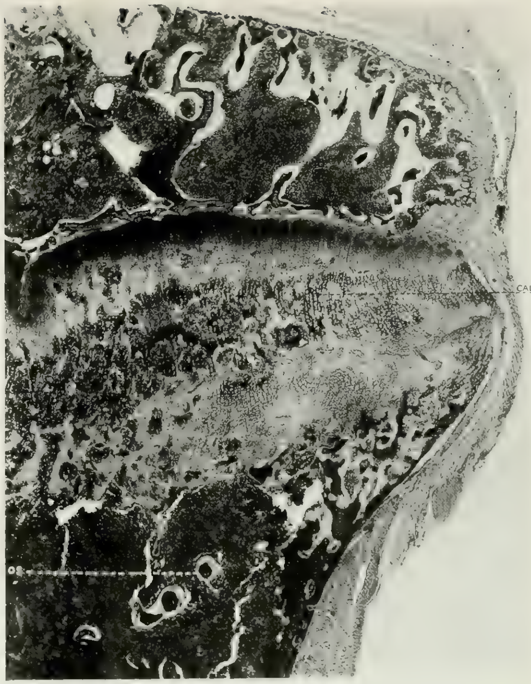

Fig. 10.

Fig. 11.

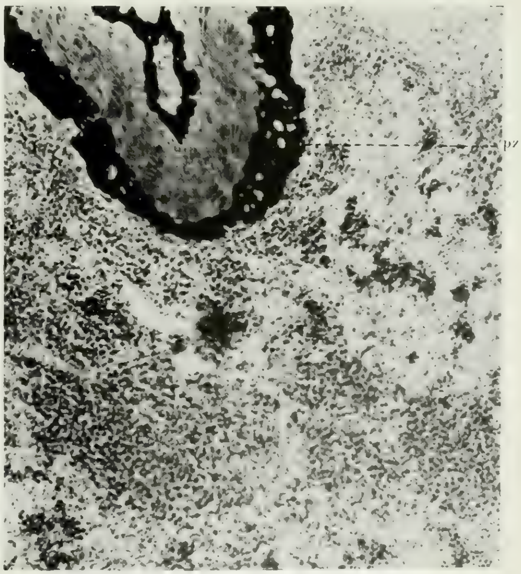

Fig. 12.

and even, the calcification of the cartilage heavy and everywhere complete, the trabeculæ thin; osteoid was not present at all, or was not in greater amount than could be accounted for by the resorptive processes, obviously in operation. The pathological condition induced in the group of rats fed this diet, deficient fat-soluble A, but containing the adequate amount of phosphorus, therefore bore no resemblance whatsoever to rickets. It showed the typical picture of osteoporosis.

The experiments recorded leave no doubt that the two diets having the known deficiencies in fat-soluble A and phosphate may produce in the bone of the rat changes having fundamental resemblance to the pathological changes in the bone which characterize rickets in the human being. At the same time they indicate that the addition of a salt mixture relatively high in its content of the phosphate ion completely prevents the development in the bone of the rat of changes resembling rickets, though it fails to exercise any preventive influence on the development of the xerophthalmia. It seems possible to infer from our experiments, therefore, that the development of rachitic-like changes in the rat induced by diets deficient in fat-soluble A and the phosphate ion can be prevented by the addition to the diet of phosphate-containing salts; and that conversely, for rachitic changes to develop in the presence of a deficiency of fat-soluble A in the diet, the diet in other respects being optimal, a low content of the phosphate ion is essential. The experiments indicate further that in the production of rickets-like conditions in the rat, fat-soluble A cannot be the sole factor concerned. Since the variable in the diets used in these experiments was certain phosphate-containing salts, it might seem possible to infer that it is necessary to consider only the inorganic content of the diet as active, and that a fat-soluble organic factor might be excluded from consideration. Even if it were granted that the induction of the rickets-like changes in the bone results from a disturbance in the level of the body's phosphate, it does not by any means follow that the inorganic ions are the only factors involved. Recent experiments in feeding cod-liver oil, which is known to be high in fat-soluble A but free from phosphate, to rats already rendered rachitic by means of the diet, have proven that an organic substance (cod-liver oil) may exert a powerful influence in causing lime salts to be deposited in the cartilage.[3] Studies on the effects of feeding cod-liver oil to rachitic children have led to similar conclusions.[4] Moreover, Howland and Kramer * have shown that the blood phosphate is low in the blood plasma of rachitic children, and that the administration of cod-liver oil causes a marked rise. It seems not unlikely that a similar result may be accomplished in rats by feeding a diet high in phosphate, and from present knowledge it seems reasonable to suppose that, while the calcification of the bones of children and animals fed diets high in their content of phosphate may be dependent on the elevation of the phosphate level in the blood, the phosphate curve may also be affected by the amount of the organic factor available for the body needs.

* Personal communication.

Finally, our experiments show beyond doubt that the addition of certain phosphorus-containing salts to the diet low in fat-soluble A in no wise prevents the development of xerophthalmia and indicates, as clinical observation has made it necessary to infer, that xerophthalmia and rickets cannot possibly have an identical etiology.

Conclusions

1. The two diets which were low in their content of fat-soluble A and phosphorus (diets of lots 2667 and 2806) produced in the majority of the young rats placed upon them pathological conditions of the skeleton having a fundamental resemblance to rickets. The pathological conditions produced are not identical, however, with that disease as it usually manifests itself in the human being.

2. The chief difference consisted in the presence of scattered or irregular deposits of calcium salts in the cartilage and metaphysis. The pictures bore a marked resemblance to those seen in rachitic children in whose bones incomplete healing has taken place.

3. When the deficiency in phosphorus is compensated for by the addition of a complete salt mixture containing the phosphate ion (diet of Lots 3120 and 2815), the deficiency in fat-soluble A still existing, no pathological changes of a rachitic nature developed. The addition of the phosphate ion to the diets deficient in it and in the organic factor prevented, therefore, the development of any changes of a rickets-like nature.

4. The experiments reported are not sufficiently numerous or comprehensive to permit of generalizations concerning the effects of a deficiencies of the fat-soluble A and phosphorus or of the fat-soluble A alone. The implication of the experiments is, however, plain.

(a) The phosphate ion in the diet may be a determining influence for or against the development of rickets.

(b) If the phosphate content of the diet is sufficiently high a deficiency of fat-soluble A cannot cause rickets-like changes in the skeleton.

(c) A deficiency in fat-soluble A cannot be the sole cause of rickets. Conversely, it is necessary that the diet be low in its content of phosphorus, all other factors, except fat-soluble A, being optimal for rickets-like conditions to develop.

5. Since the addition of the phosphate ion to the diet prevented the development of the rickets-like changes in the skeleton, but had no effect in preventing xerophthalmia, it seems permissible to infer that xerophthalmia and rickets do not have an identical etiology.

6. The above results do not in our opinion exclude the fat-soluble A from consideration as an etiological factor in the production of rickets and kindred diseases, since the level of the blood phosphate is, in all probability, determined in part by the amount of the fat-soluble A available for the needs of the organism.

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4. Howland, John, and Park, E. A.: Arch. of Pediatrics, 1920, XXXVII, 411.

DESCRIPTION OF PLATES

Fig. 1.—Microphotograph showing advanced rickets-like changes in the lower end of the left femur. The picture shows the relative positions of the epiphyseal nucleus (e. n.), cartilage (c), the metaphysis (Met.) and the diaphysis (d) of the bone. The epiphyseal cartilage is persistent at the periphery of the bone.

Fig. 2.—Microphotograph of the lower end of the left femur showing marked rickets-like condition.

Fig. 3.—High power photograph of the epiphyseal cartilage—extreme rickets-like lesion showing the arrangement of the cartilage cells in the matrix.

Fig. 4.—Microphotograph of the epiphyseal cartilage from a rat showing a marked rickets-like condition, to show persistence of the cartilage cells and the degenerative changes in them.

Fig. 5.—This picture shows the cell division in cartilage cells which are in contact with tissues of the metaphysis. The cell capsule (c) contains four daughter cells which have arisen by the division of the cartilage corpuscle which originally occupied the capsule.

Fig. 6.—Trabeculæ in the medullary cavity of a rickets-like bone. The small spicule (o) which has undergone calcification is surrounded by a broad zone of osteoid tissue (o. s.).

Fig. 7.—This picture shows the sub-periosteal hyperplasia of the osteoid tissue—the so-called rachitic periostitis.

Fig. 8.—An abortive attempt at healing an extreme rickets-like lesion is recorded in the metaphysis of the affected bone by the linear deposit (l.) of calcium salt.

Fig. 9.—Microphotograph of a bone in which the metaphysis showed traces of three periods of calcium deposition, which are in all cases incomplete or have been partially absorbed.

Fig. 10.—This picture from a section of a bone which was in the transitional stage between the rickets-like condition and osteoporosis shows irregularity and prolongation of the epiphyseal cartilage, and its irregular calcification (cal). Broad zones of osteoid tissue (os) still persist about the trabeculæ.

Fig. 11.—The trabeculæ shown in this figure are further along in the process of absorption than those shown in Fig. 10. Calcification of the cartilage is incomplete in this bone. Osteoid tissue (os) is still present in the diaphysis.

Fig. 12.—Microphotograph showing the cartilage and part of the medullary canal of a bone from a rat maintained on a diet which included a complete salt mixture (185) high in phosphorus. This bone was extremely osteoporotic. The proliferative zone of cartilage (pz) is narrow and was completely and heavily calcified, as was the cortex. There were no trabeculæ in the medullary cavity, and no traces of osteoid tissue to be found in the diaphysis.

GIARDIA (LAMBLIA) INTESTINALIS

A COMMON PROTOZOAN PARASITE OF CHILDREN

By Kenneth F. Maxcy

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Studies of the protozoa found in the intestine of man received a great impetus during the war, due chiefly to the return of large numbers of soldiers from the war area suffering from chronic diarrhœas and dysenteries of various sorts. In investigating the underlying cause of these conditions, a surprisingly high percentage of infestations not only with Entamœba histolytica but also with supposedly non-pathogenic protozoa was disclosed. This led to the examination of non-dysenteric soldiers, of soldiers who had not been outside the country and, finally, of the civilian population in the community at large, with the result that approximately these same percentages were found to hold for all four groups. Pooling the results of all the recent surveys in England, France and the United States, Hegner and Payne [1] find the percentages of infestations with the three commonest intestinal protozoa to be as follows: Entamœba coli 20 per cent, Giardia (Lamblia) intestinalis 12 per cent, and Entamœba histolytica 9 per cent. This work has thrown an entirely different light upon the problem of amœbic dysentery and upon the so-called "flagellate" dysenteries and diarrhœas, of which occasional mention is found in medical literature. It also suggests a reconsideration of the protozoa as intestinal parasites widely distributed in man.

The frequency of Giardia is particularly striking inasmuch as this parasite of man has received little attention in the United States. Although Lambl first described it in 1859, we are really indebted to Grassi for most of our early knowledge concerning the organism. In the early eighties of the last century, he made a careful study of it and demonstrated its habitat to be the upper intestine, where it might be found in huge numbers perched upon the lining epithelium, to which it adhered by means of a sort of sucking disc. He observed a number of individuals who harbored the parasite and thought that in certain instances it was the cause of diarrhœa.

A little later, 1892, Moritz and Hölzl [2] published a lengthy paper on the infestation of man with Giardia, reporting observations on six patients with diarrhœa and upon finding the organism in the duodenum of ten patients dead of tuberculosis. They noted the frequency with which the parasite occurred in children.

Since these early studies, there have appeared in medical literature a large number of reports of the finding of Giardia in the stools of patients, with or without an accompanying diarrhœa. In the United States attention was first called to it by Stiles [3] in 1902, reporting the case of a patient of Dr. Hemmeter of Baltimore, a three-year-old white child with recurring

[1] Hegner, R. W., and Payne, G. C. Scientific Monthly, 1921, XXI, 47-52.

[2] Moritz and Hölzl: Münch. med. Wchnschr., 1892, XXXIX, 831.

[3] Stiles, C. W.: Washington Medical Annals, 1902, I, 64.

attacks of diarrhœa whose stool contained large numbers of the motile organism. In 1910 Allen[4] reported a similar condition in a five-year-old colored girl. Two years later, in an article by Dubois and Toro,[5] we find an account of four children infested with Giardia.

Emerson,[6] in his text-book, mentions a case discovered in the wards of The Johns Hopkins Hospital. In the Public Health Service Reports of 1911, Stiles[7] makes the statement that Giardia is by no means rare in the United States and says that he encountered it in a number of instances in the course of fecal examinations for parasitic worms in North Carolina. Among 6000 admissions to the Mayo clinic, Giardia was found in 66 or a trifle over 1 per cent,[8] whereas among 1000 admissions to Augustana Hospital in Chicago it was recorded in only five instances, or 0.5 per cent.[9] Finally, in a very careful study of 1500 soldiers of the United States Army, 300 of whom had not been overseas, for intestinal parasites, Kofoid, Kornhauser and Plate[10] report 6 per cent infested with Giardia.

It will be noted that many authors have regarded this organism as pathogenic—that is, as a cause of diarrhœa. This conclusion is usually based upon the presence in the diarrhœic passages of a large number of motile Giardias which apparently disappear with improvement in the clinical condition. In one or two instances examination of tissues removed at operation or after death has seemed to support the contention. In areas where the surface epithelium had been denuded, these protozoa seemed to have penetrated into the wall of the intestine. On the other hand, many have denied it any pathogenic rôle whatsoever, contending that it was present in diseased conditions of the intestine, not as the primary pathogen, but as a secondary invader, that it deserved no more consideration than is given to Entamœba coli, for instance.

Clinical-pathological studies so far published have, then, failed to solve the problem, and attempts have been made to attack it from the experimental side. The method has consisted mainly in inoculating rabbits,[11] guinea-pigs,[12] and rats,[13, 14] with Giardias obtained from man. This method is complicated by the fact that these rodents are in nature infested with their own species of Giardia: Giardia cuniculi in the rabbit, Giardia muris in culture mice and culture rats, and Giardia microti in the meadow mouse, etc. These species are more or less similar in appearance to that found in man and can be distinguished only after careful morphological and biometrical study, and observations on the original habitat. If rodents are to be used in inoculation experiments, natural infestation must be carefully excluded (a feat very difficult of accomplishment), and the origin and classification of the strain of Giardia used in the experiment must have been carefully determined. These and other considerations render previous experimental work on the pathogenicity of Giardia inconclusive. For similar reasons, the statements which have crept into the literature regarding rodents as a reservoir of infection for man have not been firmly established.

To recapitulate, it may be said that previous work has demonstrated that Giardia intestinalis is a widely distributed intestinal parasite of man whose power to harm its host has been strongly suspected but is as yet unproved. Assuming that it is capable of harm, then this should be most apparent at the age when the intestinal tract is most sensitive to disturbing influences of all kinds,—namely, in childhood. The literature lends some support to this hypothesis in so far as many of the cases of so-called "Lamblia enteritis" which have been reported have been in children. The problem formulated is, whether Giardia intestinalis is simply a harmless commensal in children or whether it is capable of causing intestinal disturbance.

As a contribution to the solution of this problem the present study was undertaken to determine the frequency of this parasite in the intestinal tract of apparently normal children in the United States. It is obvious that this information is necessary before its presence in those with abnormal intestinal conditions can be properly evaluated.

Methods and Material

Protozoa are apt to be missed in the ordinary clinical laboratory examination of feces. Specimens are rarely examined immediately after passage, although it is known that the actively motile protozoa die and undergo lysis a few hours after they have left the body of the host. Again, the microscopic examination of feces is often carried out with the low power objective which gives a sufficiently high magnification for the detection of helminth ova; but the detection of the encysted forms of the protozoa require more careful examination, often of several preparations, and with the use of the higher magnifications.

In pursuing the present study, attention was directed toward demonstrating only the encysted Giardia, and incidentally the other common intestinal protozoa which show encysted forms; *viz.,* Entamœba coli, Entamœba histolytica, Endolimax nana, Chilomastix (Tetramitus) mesnili, Coccidia and Blastocystis.

The specimens were obtained for the most part without the previous administration of a cathartic, and were usually formed stools. Two "wet smear" preparations were made from each specimen, distilled water being used. Material for the one was scraped from the exterior of the stool, any clumps of mucus visible being included; material for the second was taken from the interior. A drop of one per cent water soluble eosin was added to the preparation. The field takes a pink stain; the viable cysts remained unstained and stand out rather

[4] Allen, Wm.: Old Dominion Jour. Med. & Surg., 1910, X, 417.

[5] Dubois, E. F., and Wm. C. Toro: Proc. N. Y. Path. Soc., 1912, XII, 32.

[6] Emerson, C. P.: "Clinical Diagnosis," Phila., 2d ed., 1908, pp. 405-406.

[7] Stiles, C. W.: U. S. P. H. S. Reports, 1911, XXVI, 1347.

[8] Logan and Sanford: Jour. Lab. & Clin. Med., 1916-17, II, 618-621.

[9] Smithies, Frank: Am. Jour. Med. Sci., 1918, CLVI, 173.

[10] Kofoid, Kornhauser and Plate: Jour. A. M. A., June, 1919, 72: 1721.

[11] Perroncito: Centralblatt f. Bakt., 1887, II, 738.

[12] Stiles: U. S. P. H. S. Reports, Reprint No. 135, 1913, p. 89.

[13] Fantham and Porter: Brit. Med. Jour., 1916, 2900, II, 139-141.

[14] Yakimoff: Compt. rend. Soc. biol., Paris, 1918, LXXX.

sharply as clearly defined, pale green, refractive ovals against the pink background. Whenever cysts were found to be present, another preparation was made and stained with iodine (iodine 1 part, potassium iodide 2 parts, distilled water 100 parts). This solution differentiates the structures a little more clearly and brings out the iodophilic inclusions.

About 15 minutes were spent in searching through each preparation before it was discarded as negative. An 18 mm. cover-slip was used, and the field was traversed eight times in each direction, guided by a mechanical stage. The "high dry" objective (4 mm.) with a No. 10 B. & L. eye piece gave an approximate magnification of 450 ×.

When possible each patient was examined on two occasions, at intervals of two or more days. Actually the number of examinations averaged two per case. According to Dobell,[15] Wenyon and O'Connor[16] and others, this technique would reveal about two-thirds of the actually existing infestations with intestinal protozoa. This figure probably indicates the percentage of Giardia infestations detected in the present study.

The children examined were miscellaneous medical admissions to the clinic of Dr. John Howland, Harriet Lane Home, Johns Hopkins Hospital. Excepting a small group of "feeding cases," these children were admitted because of acute infectious disease, and were free from gastro-intestinal disturbance. They were fairly representative of the child population of Baltimore with the exception that the negro race was not proportionately represented (only 6 in the 89 were colored).

Results

Of the total of 89 children examined, 18, or 20 per cent, were found to be harboring intestinal protozoa of some type. It is noteworthy that in a group of 15 "feeding cases" under one year of age no infestations were found. The youngest positive case was a little girl 17 months of age whose stools showed large numbers of Entamœba coli cysts. From the second year on there was an increasing number of positive cases with advancing age, so that the group of children from 6 to 12 years old showed a decidedly higher percentage of infestations than the children between one and five.

The relative frequency of the protozoa encountered in this series and the instances of multiple infestations are shown.

Giardia intestinalis (alone)	10 cases
Giardia and Entamœba coli	1
Giardia and Blastocystis	2
Giardia, Hymenolepis nana and Oxyuris vermicularis	1
Entamœba coli (alone)	4
	18

The most interesting fact brought out by this study was the large number of children who harbor Giardia intestinalis. If the infants under one year are excluded, then of the remaining 74 children, 14 were infested with this parasite. In other words one out of every five of the children over one year of age examined harbored Giardia. The raw data indicating the sex and age distribution of these children are given in the accompanying table. The group is in itself too small to admit of conclusive analysis, but may prove of value in connection with other reports of a similar character in the literature.

TABLE 1.—AGE AND SEX DISTRIBUTION OF CHILDREN FOUND TO BE INFESTED WITH GIARDIA (LAMBLIA) INTESTINALIS

Age	Males 58		Females 31		Total 89	
	Non-infested 52	Infested 6	Non-infested 23	Infested 8	Non-infested 75	Infested 14
0— 1	12	0	3	0	15	0
1— 2	10	0	4	1	14	1
2— 3	6	1	5	0	11	1
3— 4	9	0	2	1	11	1
4— 5	5	2	1	2	6	4
5— 6	2	0	3	1	5	1
6— 7	2	0	1	1	3	1
7— 8	0	2	1	1	1	3
8— 9	1	0	0	1	1	1
9—10	1	0	1	0	2	0
10—11	1	0	0	0	1	0
11—12	1	1	2	0	3	1
12—13	2	0	0	0	2	0

The youngest instance of Giardia infestation was in a little girl 22 months old. In the group of one to five years of age, 17 per cent showed Giardia, and from 6 to 12 almost 40 per cent were positive.

The history of each one of these positive cases was obtained. None had suffered from chronic diarrhœa so far as could be learned. One gave a history of a rather severe diarrhœa in the preceding summer, but such a history might be expected in at least one of a group of this size, and no conclusions as to its etiology could be drawn. Three of the children showed a tendency toward increased frequency of defecation—three or four times daily. This may be a significant observation, as it is what one might expect from duodenal irritation.

Case 38, a child five years old, had on admission a diarrhœa. The passages were dark brown in color and contained a large amount of yellowish mucus in which were entangled myriads of motile Giardia. The condition, however, cleared quickly and after two or three days only the encysted forms could be found in the soft, semi-formed stools, which, however, still contained some mucus at times. No history of repeated attacks of diarrhœa was obtainable in this individual.

The intermittency with which Giardia cysts are discharged was particularly well illustrated by two of the series. In case 19, examinations on January 27, 29 and 31 were negative; on February 3 and 5 large numbers of Giardia were found. In Case 37, the examination on February 24 was positive. No cysts were found on February 27, 29 and March 4. On March 6 large numbers of cysts were again found and were still present at the last examination on March 11.

[15] Medical Research Committee, National Health Insurance, London. 1918, Special Report Series Nos. 7 and 15; 1921, No. 59.

[16] Wenyon and O'Connor, J.: Royal Army Med. Corps, 1917, XXVIII, 1-24.

Discussion

This intermittency emphasizes the necessity of repeated examinations for the establishment of a negative result, in order to judge of the heaviness of the infestation or the permanency of a cure. This has been pointed out by many observers. An attempt has been made to show that Giardia undergoes a regular cycle of cyst production. Porter [17] made daily examinations of a group of soldiers infested with Giardia and estimated the number of cysts passed in 24 hours. The number varied within wide limits, being practically nil on some days and more than 300,000 on other days. She estimated the interval between successive maxima and minima at about 14 days. Boeck,[18] working with the Giardia microti of the meadow mouse, found a similar phenomenon but fixed the interval at seven days. Dobell,[19] on the other hand, finds absolute irregularity in the passage of cysts, suggesting that there may be prolonged periods of complete quiescence during which no cysts can be found.

At all events, it is plainly evident that a single examination or two examinations reveals only a part of those actually parasitized. In the series here reported, while Giardia was actually found in one out of every five children examined, a conservative estimate would place those actually infested at close to one in four.

Results of British investigations [20] indicate a similar high degree of incidence of Giardia in the intestinal tract of children in that country. Matthews and Smith examined 548 children in the Liverpool Royal Infirmary, and, based upon a single examination per case, found 14 per cent infested with this protozoon. A. H. Campbell found 16 per cent positive in a group of 49 children under 12 years of age in the Children's Hospital, St. Michael's Hill, Bristol.

Muriel M. Nutt examined 128 children in Leeds, England. Among a group of children living in an institution (Workhouse), 48.8 per cent were found to harbor Giardia as compared with 23.9 per cent of the children in the Leeds General Infirmary. The higher incidence among inmates of an institution is a significant fact. In the Sheffield Royal Hospital, this same observer found 15.8 per cent of 57 children examined positive for Giardia.

Most of these figures are based upon a single examination per case. It seems to be clearly established that in England Giardia is a very common intestinal protozoon in children. Our own studies indicate that the same thing is true in the United States.

How soon the infestation is acquired is a question of considerable interest and importance. It has been noted that no cases were found among the 15 infants under one year of age examined. Matthews and Smith found none among the 50 infants which were examined by them. On the other hand, Miss Nutt found no less than 6 of the 25 children under 12 months of age examined by her to be harboring Giardia. The ages were respectively: one at three weeks, one at three months, one at nine months, one at eleven months and two at twelve months. That an infant three weeks old may become infested with Giardia is a startling fact, and indicates how soon the food of children in filthy surroundings becomes contaminated with human fecal material. However, this must certainly be an exceptional case, and infestation during the first year of life is a comparatively rare event.

Sufficient figures are not yet at hand to determine at what year of childhood the maximal incidence is found, but it is not unlikely that it is before the fifth year as is the case with Oxyuris vermicularis. Indeed, these two parasites may be transmitted from child to child in much the same way. Experimental work has demonstrated that Giardia cysts can remain alive outside the human host for only a few hours except under very favorable circumstances as regards moisture and absence of other deleterious influences. If allowed to dry, they perish immediately. An intermediate host has not been shown to exist nor to be necessary. The obvious inference on the basis of our present knowledge is a rather direct transference by fecal contamination of fingers and food. Without doubt it is just one more of the unwelcome guests along with the tubercle bacillus and metazoan parasites which children pick up during the " dirty age."

How long the infestation may last is as yet undetermined. This much is certain: A very much higher incidence is detected in children than in adults. The British figures are very consistent on this point. In the same population groups, the percentage of adults showing Giardia cysts is from one-third to one-fifth that of the children. In this country, as previously mentioned, Kofoid, Kornhauser and Plate found 6 per cent of the healthy, young adult soldiers who had never been outside the United States harboring Giardia. If this figure is compared with the 20 per cent of children found infested in this investigation, the same discrepancy is apparent.

The explanation suggested is that in a certain number of children the infestation is not permanent, and that there is either a heightened immunity or lessened exposure in adult life. The same phenomenon has been noted in the protozoon infestations of certain animals, namely, that the young of the species show a far greater degree of infestation than the adults of the same species. Here the lessened exposure is not so obvious and greater emphasis must be laid upon factors of immunity. These latter may simply be changes in the bacterial flora of the intestine or in food habits. To these views Dobell [20] objects that it has not yet been demonstrated in any particular instance that an individual once infested with Giardia has subsequently been ridded of the parasite. Absence of the cysts in the stools does not necessarily mean that the organism is no longer present in the intestinal tract. Cyst production may be in abeyance in adults. He has himself observed an infected individual over ten years, and although it is

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[17] Porter, Annie: Lancet, London, 1916, I, 1166-69.

[18] Boeck, Wm. C.: Univ. of Cal. Pub., Berkeley, 1919, XIX, 85.

[19] Dobell, Clifford: Lancet, London, 1916, II, 1053.

[20] Medical Research Council, London, 1921, Special Report Series, No 59.

frequently impossible to find the organism in the stools the infection is still present and has shown no disappearance from the bowel. Obviously it is just as wrong to generalize from this one case, and until it has been proved that Giardia is as frequent in the intestinal tract of adults as in children, it may be assumed that a certain number of children do get rid of their Giardia infestations.

As regards the pathogenicity of Giardia, in view of the large number of apparently normal children harboring the organism the conclusion is almost forced that in a vast majority of instances it is present merely as a harmless commensal. On the other hand, the fact that, in at least one of the 14 positive cases found in this investigation, there was evidence of an intestinal disturbance which could possibly be attributed to Giardia makes necessary guarded inferences as to its entire harmlessness in children—particularly in very young children.

There seem to be authentic instances in the literature in which individuals harboring this parasite have been subject to recurrent attacks of diarrhœa, during which they passed large quantities of yellowish mucus in which occur the unencysted Giardia in myriads, and in whom no other explanation for the diarrhœa is obvious. As Wenyon and O'Connor [21] point out, it is difficult to explain the large quantity of mucus or the occasion of the attacks and, above all, the crowding of the mucus with flagellates, without assuming that the mucus must be produced by the intestine at the site of the Giardia infection and that the flagellates are directly responsible for its production.

Before this parasite is ruled out as of no importance to children, a larger experience is required. Its presence or absence should be recorded in a large series of children suffering from nutritional difficulty, intestinal "indigestion," abnormal frequency of defecation, diarrhœa, etc., and compared with a like group of children, normal in these respects, and living under the same surroundings.

On the basis of this recent knowledge it is evident that the mere finding of a considerable number of Giardia in the stools of a child suffering with diarrhœa and dysentery does not necessarily mean that Giardia is the cause of the condition. In fact it would be strange if a great many such cases were not found. Misdirected efforts at therapy should also be avoided. There is no evidence at the present time that any of the innumerable drugs that have been used are of the slightest value in ridding the patient permanently of his infestation.

Conclusions

1. Giardia intestinalis is present in the intestinal tract of a large percentage of apparently normal children.

2. It is rarely found before the first year.

3. The percentage of infestations appears to be much higher in childhood than in adult life.

4. The finding of a large number of motile Giardia in a diarrhœa or dysenteric stool is not sufficient evidence to establish the etiologic relationship of this parasite.

5. In certain rare instances the parasite may be responsible for some intestinal disturbance, although this point has not yet been firmly established.

[21] Wenyon and O'Connor: "Human Intestinal Protozoa in the Near East," Wellcome Bureau of Scientific Research, London, 1917.

NOTES ON NEW BOOKS

The Form and Functions of the Central Nervous System. An Introduction to the Study of Nervous Diseases. By Frederick Tilney, M. D., and Henry A. Riley, M. D. Cloth, $12.00. (*New York, Paul B. Hoeber, 1921.*)

In one volume of a thousand pages the authors have combined the important data which are indispensable to a proper understanding of clinical neurology. No laborious attempt to correlate a mass of anatomical facts by doubtful significance has been made. The importance of phylogenetic investigations in relation to the functions of the nervous system of man has been clearly stated.

The work is of particular value to students and clinicians, since it contains much information not available in text-book form in English. Furthermore, that information is clearly set forth in a rational manner. The microphotographs and drawings are in most instances well reproduced. A few of the former are so dark that detail is lost. Very few authors are cited in the text, but a fairly complete bibliography is appended.

The field of usefulness for the book exists and its appearance should be welcomed. V. R. M.

Physiology and Pathology of the Cerebrospinal Fluid. By William Boyd. Cloth. (*New York: MacMillan Co., 1920.*)

In his preface the author states that "the object of this book is to present some of the fascinating physiological problems connected with the cerebrospinal fluid, and to show how they are related to the pathological problems which more directly concern the clinician." This object is attained. The subject is presented from a physiological standpoint in a clear, if somewhat elementary, fashion. The more recently discovered facts pertaining to the origin and circulation of the cerebrospinal fluid, and the finer anatomical relationships of the subarachnoid space are brought together and correlated in Part I.

Part II of the book deals with the cerebrospinal fluid in special pathological conditions, and a section on therapeutics is herein included. It is to be regretted that Part I was not elaborated in more detail—to the complete exclusion of Part II from the volume. An excellent bibliography is given. H. M.

The Mechanism and Graphic Registration of the Heart Beat. By Thomas Lewis, M. D. Cloth, $16.00. (*New York: Paul B Hoeber, 1920.*)

Although advance in special methods of cardiac study has been remarkably rapid in the last few years, one cannot but feel that in a way the crest of the wave has been reached. General principles have been pretty well established, and it now remains mainly to work out the finer details of the disturbances of cardiac mechanism Another edition of Lewis's masterly treatise seems, therefore, most timely, bringing up to date, as it does, the whole subject. As those familiar with the previous editions know, the subject is taken up mainly from the standpoint of pathological physiology. The authority of the writer and the constant references to his own work do not interfere with a critical discussion of the literature in general, so that the book stands out as a storehouse of information in this large and complex domain of medical science. A. L. B.

BOOKS RECEIVED

Scurvy, Past and Present. By Alfred Hess, M.D. Illustrated. 1920. 8°. 279 pages. J. B. Lippincott Company, Philadelphia and London.

A Handbook of Midwifery. For Midwives, Maternity Nurses, and Obstetric Dressers. By Comyns Berkeley, M. A., M. C., M. D. (Cantab.), F. R. C. P., Lond., M. R. C. S., Eng. Fifth edition, enlarged with color frontispiece and 74 illustrations in the text. 1921. 16°. 550 pages. Paul B. Hoeber, New York.

Insects and Human Welfare. An Account of the More Important Relations of Insects to the Health of Man, to Agriculture, and to Forestry. By Charles Thomas Brues. 1920. 8°. 104 pages. Harvard University Press, Cambridge.

The Basis of Psychiatry (Psychobiological Medicine). A Guide to the Study of Mental Disorders for Students and Practitioners. By Albert C. Buckley, M. D. 79 illustrations. 1920. 8°. 447 pages. J. B. Lippincott Company, Philadelphia and London.

Old at Forty or Young at Sixty. Simplifying the Science of Growing Old. By Robert S. Carroll, M. D. 1920. 12°. 147 pages. Macmillan Company, New York.

Surgery of the Lung and Pleura. By H. Morriston Davies, M. A., M. D., M. C. (Cantab.), F. R. C. S. (Eng.), Hon. Captain R. A. M. C. (T.). 1920. 8°. 259 pages. Paul B. Hoeber, New York.

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Tropical Ophthalmology. By Robert Henry Elliot, M. D., B. S. (Lond.), Sc. D. (Edin.), F. R. C. S. (Eng.). With 7 plates and 117 illustrations. 1920. 8°. 525 pages.

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A CRITICAL ANALYSIS OF TWENTY-ONE YEARS' EXPERIENCE WITH CÆSAREAN SECTION

By J. Whitridge Williams *

From February 1, 1899, until the end of December, 1920, 183 Cæsarean sections were done in the Obstetrical Department of The Johns Hopkins Hospital, and I have thought that it might be interesting to follow the evolution of the operation in our service, and to learn our point of view concerning certain of its various aspects.

Frequency

The 183 operations were done upon 145 women, 104 of whom had their first and only section in the service, while 41 others had one or more subsequent sections, as follows:

104 women had the first and only section in the service	104
8 women had the first section elsewhere and the second in the service	8
26 women had two sections in the service	52
2 women had the first section elsewhere and two in the service	4
5 women had three sections in the service	15
145	183

Of the entire number of operations, 122 were done by me and 61 by a succession of 13 residents. Accordingly, the results obtained are composite in character, and do not represent the experience of a single, and possibly peculiarly skillful, individual.

As the 183 sections occurred in a series of approximately 20,000 deliveries, their incidence was somewhat under 1 per cent. As will be shown below, 144 of them were done on account of disproportion between the size of the child and the pelvis, and I believe that great conservatism was observed in the indications for its performance. Quite one-half of our material consists of colored women, and as the incidence of contracted pelvis is 35 and 8 per cent in the two races, respectively, this means that approximately 3500 contracted pelves were observed in our black and 800 in our white patients, or a total of over 4000 in the series. Dividing that number by 144 shows that during a period of 21 years approximately one Cæsarean section was done in every 30 contracted pelves. As time has gone on, our indications have become somewhat more liberal, as is shown by the fact that only 50 sections were performed during the first 12 years of the period, as compared with 133 during the last 9 years—an average incidence of 4 and 15 sections per year, respectively.

The comparatively infrequent resort to Cæsarean section during the former period is in part accounted for by the fact that between the years 1906 and 1915 we gave pubiotomy an extensive trial. Forty-three such operations were done and

* Read in abstract as the Jerome Cochran Lecture before the Medical Association of the State of Alabama, April 20, 1921.

all of the patients recovered; and, as will appear later, pubiotomy, at that time at least, constituted a very conservative procedure, no matter what one may now think of its justifiability and applicability. That many of these patients would otherwise have been delivered by Cæsarean section is shown by the fact that 11 patients in the present series had been subjected to one or more pubiotomies before Cæsarean section was resorted to, and that three of them required two and one three sections afterwards.

One of the striking features brought out by the consideration of our series of sections is that blacks require radical interference much more frequently than whites, our figures showing that Cæsarean section was performed upon 114 colored and 69 white women, with 57 and 14 repeated operations in the service, respectively—an incidence of 50 and 20 per cent. To particularize, it may be stated that 21 of the black women had two, and five had three repeated sections in the service; while none of the 7 white women had a third section in the service, although one of them (8955) had two sections following a previous section elsewhere, thus making three in all. It will be noted that these figures do not tally with those given at the beginning of the article, for the reason that they do not include 8 patients upon whom a second section was done in the service following a first one performed elsewhere.

Considering the types of operation performed, it is found that 121 were typical conservative sections, 4 extraperitoneal sections (Küstner), 1 postmortem section, and 57 supravaginal amputations of the uterus—the so-called Porro Cæsarean sections. It will be noted that no cervical Cæsarean sections were done. Of the 125 conservative and extraperitoneal sections 27 were repeaters, as compared with 21 in the 57 supravaginal amputations. Moreover, the comparatively frequent employment of supravaginal amputation of the uterus following the section should lend considerable interest to our analysis, and the reasons for its frequent utilization will be considered in some detail further on.

Mortality

Passing to the consideration of the mortality following Cæsarean section, it is found that 10 deaths followed the 183 operations, a gross mortality of 5.46 per cent; eight deaths occurring after the 126 conservative operations, and two after the 57 supravaginal amputations, or 6.35 per cent and 3.51 per cent, respectively. Before any deductions are made, it should be noted that the gross mortality following supravaginal amputation, which for purposes of brevity will hereafter be designated as the Porro operation, was about one-half of that following the conservative operation.

Careful study of our material has convinced me that a gross mortality of 5.46 per cent gives somewhat too sombre an idea of the dangers of Cæsarean section, and it will be seen that the deaths, which followed the operation in several instances, were in no way connected with it, and may be fairly deducted in determining the net mortality. For example, Case 4723, in which the patient with mitral stenosis and insufficiency was operated upon in the hope of preventing death from acute decompensation, made a good recovery from the operation, but died at home forty days later from the original heart lesion. Likewise, in Case 5709, the woman was suffering from broken compensation due to acute ulcerative endocarditis, and was operated upon as a last resort with a pulse of 136. She died on the eighth day, but autopsy showed that death had resulted from the original endocarditis and that no signs of wound infection were present. In Case 5911 the patient was an elderly primipara, who was admitted to the service with complete placenta prævia, a rigid cervix and advanced chronic nephritis. Death occurred on the eighth day following the section. Autopsy showed that it was due to the chronic nephritis; while negative cultures from the uterine and peritoneal cavities, as well as the absence of inflammatory lesions, demonstrated the absence of wound infection. Finally, in Case 6890, the patient was operated upon for eclampsia. Autopsy, 26 days after the section, showed that death had resulted from acute anemia following profuse gastric and intestinal hemorrhage, following the erosion of a duodenal ulcer.

Upon deducting these four deaths, which can fairly be claimed to have no connection with the operation itself, it follows that six deaths, which must be attributed to it, occurred after 179 Cæsarean sections—a corrected mortality of 3.35 per cent. Furthermore, it is interesting to note that the net mortality following the 121 conservative and 56 Porro operations presented variations similar to those observed in the gross mortality; as five deaths occurred after the former, as compared with one after the latter—a net mortality of 4.07 and 1.82 per cent for the two operations, respectively.

A good deal of light may be thrown upon the mortality, and the factors concerned in its production by considering our material from another point of view. Regarding the first 50 operations as representing the period of learning and the remainder as the period of more ripened experience, it is found that 6 deaths occurred in the first, as compared with 4 in the succeeding period—a gross mortality of 12 and 3 per cent, respectively. Upon deducting the four deaths, which were not primarily connected with the operation, it is found that five deaths occurred in the first 49 sections, as compared with one in the succeeding 130—a net mortality of 10 and 0.77 per cent, respectively. In other words, the net mortality was 13 times less than in the second period. Moreover, during the first period we performed 28 conservative and 22 Porro Cæsarean sections, with 5 deaths in the former and 1 in the latter, a gross mortality of 18.5 and 4.5 per cent, respectively. In other words, during the period of learning the mortality was four times greater after the former than after the latter operation.

Results

It should be noted that five of the 6 deaths directly attributable to the operation were due to general peritonitis, which resulted from the extension of infection through the uterine wound, while the sixth was due to the failure of an inexperienced resident to control hæmorrhage from the uterine artery during the course of a Porro operation. As the aseptic technique was, roughly speaking, identical throughout the entire

series, the question arises as to how the difference in the results during the two periods are to be explained, and why the mortality following the Porro operation was so much lower than after the conservative? To answer these questions, as well as to ascertain what other conclusions can be drawn from our experience, is the object of the present study.

The answer to the first question is perfectly clear, and consists in the fact that we now aim to operate before the onset of labor, or as soon afterwards as is feasible, upon patients who have not been examined vaginally for some days. During the period of learning, on the other hand, the significance of early operation was not appreciated, as it was currently taught that the ideal procedure was to allow the patient to go into the second stage, to subject her to the test of labor, and to complete delivery by Cæsarean section only after a tentative attempt with high forceps had failed to draw the head into the pelvic cavity.

This lesson was in great part learned by comparison between the results following the conservative and the Porro operations. The first supravaginal amputations were undertaken for the purpose of sterilizing the patient, usually at the second section, or occasionally at the first, if she urgently requested it, or when she was so deformed as to become a charge upon the community. We soon became impressed by the difference in the convalescence following the two operations, and during the period of learning noted that only 41 per cent of the patients subjected to the conservative operation had normal afebrile puerperia, as contrasted with 71 per cent after the Porro operation, not to mention the difference in mortality to which reference has already been made. Moreover, we gradually learned that patients upon whom conservative sections were done late in labor or following vaginal manipulations did badly, and sometimes died; whereas those in whom the uterus was amputated tended to have an ideal convalescence. This contrast was particularly noticeable when signs of intrapartum infection were present prior to operating.

The more favorable results following the Porro operation were at first attributed to the lessened resistance to infection of the incised involuting uterus, and seemed explicable in the same manner as the simple convalescence following the treatment of uterine myomata by supravaginal hysterectomy as compared with the more stormy one following myomectomy. Later, however, it was found that this was not the whole story; for when we came to examine microscopically uteri that had been amputated following Cæsarean section, particularly when the operation had been undertaken late in labor, it was noted in many instances that the decidua lining the lower uterine segment presented pronounced leukocytic infiltration and other signs of inflammation, which were usually lacking in the upper part of the uterus. Moreover, when such sections were treated with appropriate stains, streptococci and other bacteria could be demonstrated in the tissues. Such observations led us to conclude that the infection was due to an ascending process, which had not given rise to clinical symptoms at the time of operation. Thus, in 14 instances in which the uterus had been amputated late in labor, microscopic study conclusively demonstrated the existence of an ascending infection. In two instances the patients had presented signs of intrapartum infection, but in the other 12 the pulse and temperature were normal at the time of operation, and no signs were present which might lead one to suppose that infection had already occurred. The evidence thus adduced is so clear that it is now realized that whenever a considerable time has elapsed between the onset of labor and the performance of Cæsarean section, we must reckon with the probable existence of latent infection. In this event, it would appear that the involuting uterus is unable to offer a sufficient resistance to the spread of the infection, with the result that the process extends through the uterine incision to the peritoneum, giving rise to a general peritonitis to which the patient almost always succumbs. Since the probability of latent infection in prolonged labor has been recognized and our procedure modified accordingly, the results following conservative Cæsarean section have become highly satisfactory.

The difference between the two operations can be still further demonstrated by comparing the clinical course of the puerperium. Upon designating as febrile all patients in whom the temperature reaches 100.5° F. on one occasion or more, the convalescence was found to be afebrile in 43.9 and 59.6 per cent following the conservative and Porro sections, respectively. Furthermore, the comparison becomes even more striking when the cases are arranged in groups according as the operation was done before the beginning of labor or at varying periods after its onset.

TABLE I. SHOWING INCIDENCE OF NORMAL PUERPERIA ACCORDING TO TIME AT WHICH SECTION WAS DONE

	Conservative			Porro		
	Normal	Febrile	Per ct.	Normal	Febrile	Per ct.
Before onset of labor	25	21	54.4	10	4	71.4
1 to 6 hours after onset....	25	28	47.2	7	5	58.3
6 to 12 hours after onset...	1	6	14.3	1	0	100
12 to 24 hours after onset..	2	8	20	4	3	57.1
Over 24 hours after onset..	1	6	14.2	12	11	52.2
	54	69	43.9	34	23	59.6
	123			57		

This table shows that there was a progressive and rapid diminution in the number of afebrile puerperia according as conservative section was done before the onset of labor or after the lapse of 24 hours—54.4 per cent as compared with 14.2 per cent. After the Porro operation, on the other hand, it is noted that afebrile puerperia are much more frequent, and while their incidence tends to decrease as the operation is performed late, it is nevertheless practically the same when the Porro operation is done after 24 hours of labor as when the conservative section is done before the onset of labor. Such observations clearly indicate that the involuting uterus

represents a *locus minoris resistentiæ,* and that its removal does away with a considerable danger of infection.

The difference in results following early and late operation on the one hand, and the conservative and Porro operation on the other, can likewise be demonstrated by considering the mode of healing of the abdominal incision.

Upon arranging our cases in four groups according as the operation was done before labor, one to twelve hours after the onset of labor, late in labor, and after the appearance of clinical signs indicating the existence of intrapartum infection, respectively, (as in Table II), it is noted that following the conservative section there is a progressive increase in the incidence of stitch abscess and actual infection of the wound, which occur three times more frequently when the operation is done late in labor than at its onset. In the corresponding groups following the Porro section, no such change can be noted, and in the 48 operations comprised in the first three groups the incidence of defective wound healing was only 4.17 per cent as compared with 13.44 per cent following the conservative operation.

Moreover, when the patients were operated upon in the presence of intrapartum infection still more striking differences were noted. Thus, in the two instances in which the conservative operation was done, both patients died before the end of the first week and were not included in the table, while of the eight in which the operation was ended by amputation of the uterus none died, but in 37.5 per cent of them the convalescence was disturbed by widespread infection of the abdominal wound.

TABLE II. SHOWING HEALING OF ABDOMINAL WOUND ACCORDING TO TIME AT WHICH CÆSAREAN SECTION WAS DONE

Time of operation	Conservative							Porro						
	Cases	Ideal	Stitch abscess	Per cent	Infected wound	Per cent	Total defective wounds	Cases	Ideal	Stitch abscess	Per cent	Infected	Per cent	Total defective wounds
Before onset.......	45	41	3	6.67	1	2.2	8.9	13	12	0	0	1	7.6	7.6
1 to 12 hours.......	62	53	5	8.06	4	6.45	14.5	15	15	0	0	0	0	0
Late in labor	12	9	2	16.67	1	8.33	25.	20	19	1	5	0	0	5
Intrapart. infec...	..	..	..	..	..	..	..	8	5	0	0	3	37.5	37.5
Total	119	103	10	8.40	6	5.4	13.44	56	51	1	1.78	4	7.14	8 93

(Does not include 7 patients who died within week of operation—6 conservatives and one Porro.)

It cannot be stated precisely why defective wound healing occurs so much more frequently following the conservative operation at the several periods, although it may be assumed that latent infection is more probably present in patients operated upon late, and, if so, it may result in soiling and subsequent infection of the abdominal wound without causing a fatal peritonitis. In the presence of intrapartum infection, on the other hand, the patients usually die from infection following the conservative operation before local signs appear in the abdominal wound; whereas after the Porro operation the peritoneum is able to take care of the infecting agents in the absence of the involuting uterus, while localized infectious processes tend to develop in the abdominal wound.

We now turn to the consideration of the factors concerned in the causation of death following Cæsarean section. As reference has already been made to the four deaths which I do not believe should be attributed to the operation itself, six others remain for consideration. One of these occurred during the course of a Porro section, while the other five followed conservative sections. The first death (1548) was due to hæmorrhage, the patient succumbing on the table as the result of failure of an inexperienced resident to control the bleeding from one of the uterine arteries. The other five were due to infection. In two the fatal outcome was due to unavoidable accidents. Thus, in Case 4116, the patient, who had had two preceding pubiotomies, was operated upon at an appointed time before the onset of labor and died five days later from general peritonitis. Afterwards, it was found that owing to a defect in the autoclave all of the dressings had been imperfectly sterilized. In Case 8826, death after the third section was due to infection following the imperfect repair of a loop of bowel, which was adherent to the abdominal cicatrix and which was wounded when the abdomen was opened.

The other three deaths, on the other hand, were due to errors in judgment and, with our present knowledge, should have been avoided. Thus, in Case 1256, a conservative section was done late in labor upon a patient with normal pulse and temperature. In Case 1611 a repeated conservative section was done 12 hours after the onset of labor upon a patient who presented signs of intrapartum infection with a temperature of 100.8°; and finally, in Case 2158, the patient was given a test of labor in the second stage and had a pulse of 116 and a temperature of 101° when operated upon.

Pelvic Indications

As has already been mentioned, 144 of the 183 sections were done an account of disproportion between the size of the child and the pelvis, while the other 39 were necessitated by non-pelvic indications, an incidence of 78 and 22 per cent, respectively.

Table III gives an idea of the various indications, and illustrates very clearly the great differences which prevail in white and colored patients, as seen in Baltimore, the pelvic indication being present in somewhat less than six-tenths of the former, as contrasted with nine-tenths of the latter. Furthermore, it is seen that in the colored women the various types of rhachitic pelvis afforded the predominant indication, as they made up 98 of the 103 contracted pelves requiring Cæsarean section in that race; while in the white women, on the other hand, only 10 of the 41 abnormal pelves were rhachitic in origin. Or, taking the entire number of sections into consideration, rhachitic pelves offered the indication for 86 per cent of the operations in the blacks as compared with 14.5 per cent in the whites. Consequently, if the application of suitable dietetic and hygienic measures should eventually lead to the

disappearance of rickets, Cæsarean section would be very rarely indicated in the black race.

In the whites, on the other hand, the most usual pelvic indication was afforded by the simple flat pelvis, which was

TABLE III. SHOWING INDICATIONS FOR 183 CÆSAREAN SECTIONS

Pelvic indications				Other indications			
Type of pelvis	Total	White	Black	Condition	Total	White	Black
Generally contracted rhachitic.	86	3	83	Eclampsia	9	5	4
Simple flat	15	15	..	Heart	8	6	2
Flat rhachitic	13	6	7	Atresia of cervix	4	4	..
Scolio-rhachitic	8	1	7	Ovarian cysts	3	3	..
Kypho-scolio-rhachitic.	1	..	1	Neglected transverse	3	3	..
Generally contracted	6	4	2	Premature separation of placenta.	2	1	1
Funnel	6	5	1	Nephritic toxæmia	2	2	..
Achondroplasic	4	4	..	Myoma	1	..	1
Kyphotic funnel	2	1	1	Ventral fixation	1	1	..
Coxalgic	1	..	1	Pregnancy in rudimentary horn.	1	..	1
Hypoplastic dwarf	1	1	..	Hour-glass contraction.	1	..	1
Oblique (luxation)	1	1	..	Placenta prævia	1	1	..
				Excessive size of child.	1	1	..
				Carcinoma of cervix	1	1	..
	144	41	103		39	28	11
		144				39	

noted in 15 instances; while not a single pelvis of that type necessitated interference in colored women. Indeed, the simple flat pelvis is the most important variety of pelvic contraction in white women, as it frequently happens that such pelves presenting so relatively long a diagonal conjugate as 10 to 10.5 cm. sometimes give rise to serious dystocia and lead to the birth of a number of dead children before the patient comes into the hands of a competent obstetrician. Furthermore, it is interesting to note that the various types of contracted pelvis, due to factors other than rickets, were observed much more commonly in the white women, notably the generally contracted, funnel, and chondrodystrophic pelves.

Non-Pelvic Indications

Passing to the non-pelvic conditions, it will be seen that eclampsia offered the indication for section in 9 instances, and was of approximately equal frequency in the two races. This is neither the time nor the place to consider the justifiability of the treatment of eclampsia by this means, although I may say briefly that it is exactly in connection with this indication that the operation is being greatly abused, and that I expect to employ it much less frequently in the future than I have in the past. With my present experience I hold that Cæsarean section is indicated in the treatment of eclampsia only when the cervix is rigid and undilated and the patient has failed to show any improvement after venesection.

The next most common indication was afforded by heart disease, which was present in eight instances—in six whites and two blacks. In each instance the patients were suffering from marked decompensation, which had failed to yield to medicinal treatment, so that the operation was undertaken as a last resort and in the hope that by avoiding the strain of the second stage of labor the life of the patient might be preserved. As in most of these cases the lesion was a mitral stenosis, which might be expected to become worse with succeeding pregnancies, it was felt that such a possibility should be obviated by a sterilizing procedure, and consequently the operation was usually terminated by amputation of the uterus or by some operation upon the tubes.

The mere enumeration of the other non-pelvic indications in the table suffices to indicate that they were of such character as not to require extended comment, except in the case of the three sections for neglected transverse presentations and the one for placenta prævia. Generally speaking, it may be said that Cæsarean section is not indicated in the former condition unless such a degree of pelvic deformity is present as to render version and extraction out of the question. In the three cases here mentioned, however, quite different conditions obtained. In each instance the patient was a multipara with a normal pelvis who had previously had a number of normal spontaneous labors, but who had been so neglected that after a long labor she had been admitted to the service with an arm protruding from the vulva, the uterus tetanically contracted, but with the child in excellent condition. Under such circumstances version and extraction were out of the question, and the alternative lay between decapitation of the live child and Cæsarean section. Furthermore, owing to the fact that the patients had been repeatedly examined by ignorant physicians or midwives, it was felt that the probability of infection was so great that if a section were done the uterus must be sacrificed. Accordingly in each instance a Porro Cæsarean section was performed and the patient made an uninterrupted recovery.

The single case of placenta prævia also deserves mention, especially as the patient is the only one in my experience who has seemed to justify the employment of Cæsarean section in connection with this complication. Of course, I have realized for years that in rare instances, when the cervix is rigid and undilated and the bleeding profuse, Cæsarean section will offer the most favorable prospect for safe delivery in complete placenta prævia. The rarity of such a combination of circumstances is shown by the fact that in the 66 cases of placenta prævia observed in the service, this was the only one in which Cæsarean section appeared indicated, and strange to say, the death which followed it constitutes one of the few fatalities associated with that complication. In this instance the patient was a very anæmic elderly primipara with advanced chronic nephritis, and, as has already been indi-

cated, died on the eighth day following the operation, the autopsy showing that death had resulted from the underlying chronic nephritis rather than from infection or loss of blood.

Generally speaking, I believe that Cæsarean section should play only a very minor part in the treatment of placenta prævia in the hands of competent obstetricians, and I contend that the use of the rubber bag will give almost ideal results. That this has been the case in our hands, is shown by the fact that only one maternal death occurred in the last 40 cases treated by that method.

Porro Cæsarean Section

Reverting to the consideration of the Porro Cæsarean section, it will be noticed that the operation has been employed relatively frequently, and constitutes nearly one-third of our total number of sections (57 out of 183). Its frequent utilization has been in great part due to one of two factors. First, that it has been followed by uniformly good results, particularly in the presence of manifest or latent infection, and second, that we have regarded it as the most efficient and safest means of effecting sterilization, more particularly as a large number of our patients are colored women of relatively low intelligence in whom we have felt that an unlimited number of repeated Cæsarean sections was not justifiable.

In considering the 57 Porro sections in greater detail, it is found that in 36 instances the uterus was removed as a primary procedure following the delivery of the child, while in 21 it was removed at a second or third Cæsarean section. The following table gives the indications for the primary operations:

	Cases
Late second stage, or manifest intrapartum infection	9
Sterilization	6
Heart disease	5
Atresia of cervix	4
Transverse presentation	3
Uncontrollable hæmorrhage	2
Rupture of uterus in service	2
Dystocia following ventral fixation	1
Pregnancy in rudimentary horn of uterus	1
Hour-glass contraction of uterus	1
Myoma of cervix	1
Apoplexy of uterus incident to premature separation of placenta	1
Total	36

I shall discuss each group as briefly as possible. In view of what has already been said, the nine cases in which the operation was performed late in the second stage of labor need little consideration, as it has already been demonstrated that in such circumstances the operation may be regarded as a life-saver. Seven of the nine uteri in this group were examined microscopically after amputation, and four of them presented positive evidence of ascending infection, so that, had the uterus been retained, the probabilities are that many of the patients would have died from infection by the end of the first week.

In the six instances in which the uterus was removed for the purpose of sterilizing the patient, the indication was offered either by the direct request of the patient or by the existence of such deformity or so low a grade of intelligence that I felt that it was a service to the state to prevent further pregnancies.

Serious cardiac decompensation was the indication for five operations (5709, 5773, 6753, 7705 and 7832). In each instance the condition of the patient was so serious that the operation was undertaken from a double point of view: first, to carry her over the present emergency, and second, to prevent the exacerbation which must inevitably occur in future pregnancies.

In four instances (1788, 2757, 5381 and 6801) the indication for amputation of the uterus was afforded by pronounced atresia of the cervix. In three patients the condition had followed gynecological operations upon the cervix which had been performed elsewhere, while in the remaining patient no clue to the etiology of the condition could be elicited. In each instance there was no visible opening in the cervical region, which might serve as a basis for a vaginal operation, but the main indication for the radical procedure was afforded by the fact that all of the women presented signs of intra-uterine infection upon admission to the service, which was afterwards confirmed by histological examination, so that it was felt that a conservative section would have seriously compromised the chances of recovery.

The three cases of neglected transverse presentation, in which the uterus was amputated in preference to decapitating a live child, have already been considered and call for no further comment (5238, 5418, 6708).

In two instances the uterus was removed on account of atony which did not yield to the ordinary methods of treatment (5051, 7072). In both patients it was intended to do a conservative section, and the uterine incision was closed in the usual manner in spite of more than the usual amount of bleeding. The uterus, however, remained so flabby and atonic, notwithstanding several injections of pituitrin and ergot, as well as vigorous mechanical stimulation, that it was felt imperative to remove it. In a third patient the uterus was amputated on account of the hæmorrhagic disassociation of the uterine muscle following premature separation of the normally implanted placenta (7190). In this instance the uterus absolutely failed to contract and presented the consistency of wet chamois-skin, so that we were compelled to remove it from the primiparous patient after all the sutures had been laid.

It will be noted that we have classed as Porro sections two cases in which intrapartum rupture of the uterus occurred in the service, and in which immediate operation was performed (6838 and 7692). In one of these rupture occurred five hours after the onset of labor in a secundipara with a slightly contracted pelvis, whose previous labor had ended spontaneously. The second rupture resulted from the impaction of an ovarian cyst in the pelvic cavity in a multiparous woman. In both instances the uterine wounds were so exten-

sive and complicated that amputation of the uterus seemed to afford the most appropriate means of meeting the situation. The two cases in which the uterus was amputated on account of a full-term pregnancy in the rudimentary horn of a bicornate uterus (2822), and on account of a large myoma of the cervix, which completely blocked the pelvic canal and rendered spontaneous labor impossible (6384), need no justification.

In another instance the uterus was removed in order to overcome the dystocia resulting from ventral fixation (Case 2479). This patient had a normal pelvis and had previously given birth spontaneously. The existence of dystocia was not recognized until late in labor, when examination showed that the anterior wall of the uterus had become "buckled" in such a manner as to prevent engagement of the head, while at the same time the uterine cavity had become divided into two compartments, the anterior of which was occupied by the feet of the child, which were in such a position as to be just beyond the reach of the hand of the operator so that version could not be effected. The uterus was amputated, on account of the extensive manipulation, and microscopic examination demonstrated that it was a wise decision, inasmuch as definite evidences of ascending infection existed.

The last primary Porro Cæsarean was indicated by the rare condition of hour-glass contraction of the uterus (Case 10840). In this instance a bag had been introduced for the purpose of inducing labor in a patient who had gone some days beyond term. After its expulsion, labor came to a standstill, and signs of intrapartum infection developed. The half dilated cervix presented swollen and œdematous margins, so that prompt delivery seemed indicated. The resident obstetrician introduced his hand into the uterus with the intention of completing manually the cervical dilatation, but found that the contraction ring was so tightly clamped about the neck of the child that it seemed unlikely that it could be delivered even after the cervix had been dilated. In view of these conditions, Cæsarean section seemed imperative, and on account of the existing infection, as well as the extensive manipulation to which the patient had been subjected, removal of the uterus appeared to be the only procedure justifiable. A live child was obtained and the patient made an ideal recovery.

The following list gives an idea of the indications for the Porro operation in the 21 cases in which the uterus was amputated at a repeated section:

	Cases
Late second stage	4
Sterilization	11
Impetuosity of operator	1
Tearing of uterine incision	1
Rupture of scar of previous section	1
Fear of rupture of scar of previous section	1
Extensive raw area up on anterior wall of uterus	1
Blocking of vulva by condylomata	1
Total	21

These cases can be disposed of briefly. In the first group, the operation was fully justified by the microscopic demonstration of an ascending infection in each of the amputated uteri. Of the 11 cases in which sterilization was the indication for the operation, the histories show that in seven the uterus was amputated at the repeated section at the direct request of the patient; while in the other four sterilization was effected at the third section upon my own initiative, as I felt that women who have had three sections had fulfilled their reasonable duty to the state.

In the case designated as "impetuosity of operator" (1548), the uterus was amputated by a former resident during my absence. The operation was performed before the onset of labor, and a small child was delivered, which might have been born spontaneously. It is interesting to note that this is the case in which the patient died from hæmorrhage on the operating table, and represents the only death in the entire series attributable to the Porro operation.

In Case 6939 the indication for removing the uterus at the second section was accidental, as we had expected to do a conservative section, but in extracting the child the lower end of the uterine incision tore downward beneath the peritoneum of the broad ligament, and so complicated a wound resulted that it seemed more conservative to remove the uterus than to attempt to repair it.

In Case 7570 the uterus was removed on account of rupture of the scar of the previous section during the eighth month of pregnancy. The details of this accident will be described by Thomas O. Gamble in an article on the "Behavior of the Uterine Cicatrix following Cæsarean section" which will shortly appear, and is of interest from two points of view—first, that it is the only occurrence of the kind in 48 women upon whom repeated sections have been done in the service, as well as in 12 other women who were delivered by the natural passages subsequent to a preceding section, and secondly, on account of the comparatively trifling symptoms associated with the accident.

In Case 8697 the uterus was removed through an excess of caution, but also at the expressed desire of the patient. In this instance infection followed the first section, which had been done elsewhere, and resulted in the formation of an extensive utero-abdominal fistula. This was eventually repaired, and necessitated an extensive resection of the uterine wall which was closed with silk sutures. The patient soon afterwards became pregnant and came to us for a second section at which we amputated the uterus with the idea that the cicatrix of the former operation might be a source of danger in future pregnancies, but when the specimen was examined microscopically no trace of the cicatrix could be found, except a fine linear scar upon its anterior wall. This specimen also will be described by Dr. Gamble.

In another instance (10758) the indication for operation was afforded by the fact that following the first section such intimate and extensive adhesions had developed between the uterine wall and the intestines that, after they had been freed, a raw area the size of the palm of one's hand remained upon the anterior surface of the uterus. As it could not be covered with peritoneum, it was felt that still more extensive adhe-

sions would result if the uterus were retained, and consequently it was amputated.

Finally, in the last case of the series (9323) the uterus was removed on account of fear of infection at the second section. In this instance the vulva was entirely occluded by large condylomatous masses bathed by purulent secretion, and it was felt that the chances of infection would be excessive if the uterus were retained.

Upon considering these various indications in a critical spirit, it may be admitted that we have perhaps employed the radical operation too freely as a means of effecting sterilization, and within the last few years we have attempted to restrict its employment for that purpose by resecting the tubes and retaining the uterus. We now ask women who request sterilization, or whom we feel should be sterilized, whether they wish to continue to menstruate or not after the last section. It they reply in the affirmative, sterilization is effected by doubly ligating the tubes and burying their uterine ends between the folds of the corresponding broad ligament; but, if they reply in the negative, I still continue to remove the uterus as the safest and most efficient method of relieving the existing situation and of effecting definitive sterility.

Possibly, a similar criticism may be made concerning certain of the cases in which the patient was sterilized on account of broken compensation. It may, however, be stated that in such cases haste is imperative, and we are able to amputate the uterus in a shorter time than is required for a conservative Cæsarean section followed by a sterilizing operation upon the tubes. Likewise, certain of the operations which were done for some of the less common indications may be open to criticism, but each such case must be judged upon its own merits, and the justifiability of the procedure must depend upon the personal judgment and experience of the operator. In general I feel, notwithstanding certain very manifest limitations, that the Porro Cæsarean section still remains the safest means of delivering the patient, and I must confess that I perform it whenever a suitable opportunity presents. It should, however, be realized that its prime indication is when Cæsarean section is required late in labor and particularly when signs of intrapartum infection are present. In such circumstances, it is universally admitted that the mortality following the typical conservative section is too high for it to be considered a justifiable procedure, and we then have to decide whether it is better to remove the uterus and thereby do away with the possibility of future pregnancies, or to perform craniotomy upon a live child. Doubtless, such a problem will be approached differently according to one's own experience and predilections, and it must be admitted that in the future such a choice may not be necessary, particularly if the claims of the advocates of the several varieties of so-called extra-peritoneal Cæsarean section are borne out; but, until they are, I shall continue to do the Porro operation in the type of cases under consideration. From my experience with the typical extraperitoneal Cæsarean section, I should certainly hesitate to resort to that complicated procedure in any instance in which the patient is presumably already infected, as I cannot conceive of more favorable conditions for the spread of infection than the extensive broad ligament wounds which are made in the course of that operation.

Evisceration of the Uterus

In studying critically the convalescence following our series of Cæsarean sections, a number of factors have been encountered which may influence its course. The first which may be mentioned is the effect upon the course of the puerperium of the old practice of eviscerating the uterus before incising it. It will be recalled that during evolution of the modern Cæsarean section the possibility of severe, or even fatal, hæmorrhage from the incised uterus was constantly in mind. For this reason it was originally the practice to attempt to control hæmorrhage by applying a constricting rubber ligature about the cervix before opening the uterus. This, of course, necessitated eviscerating the undelivered organ through a long abdominal incision. After experience had taught that the application of the ligature was not necessary, the practice of eviscerating the uterus was continued for a time so that an assistant could compress the lower segment manually, and thereby control the flow of blood through the uterine arteries when necessary.

Upon studying the histories of our patients we find that the uterus was eviscerated in 26 conservative sections prior to 1911. After that date a smaller abdominal incision was made, and the uterus was incised *in situ* and not delivered until after the child had been extracted. Upon comparing the convalescence following the two procedures, it was found that the puerperium was afebrile in 31.8 per cent of the former as compared with 51.6 per cent of the latter. Whether evisceration of the uterus ever led to the death of a patient it is impossible to state, but it is readily understood how it favored the possibility of infection, as the bulky unemptied uterus inevitably came into intimate contact with the external abdominal wall, and was likewise handled more extensively than when it is incised *in situ*. At present we eviscerate the uterus only in patients showing signs of intrapartum infection in whom the organ is to be amputated after the delivery of the child. In such cases the abdominal wound is brought together by clamps above the cervix, and by the use of gauze packs the peritoneal cavity is still further protected against contamination by the infected liquor amnii.

Since abandoning the practice of evisceration a much smaller abdominal incision has been necessary, and I usually prefer to make it below the umbilicus. My preference for this low incision, rather than for the high one advocated by Asa B. Davis or for one extending midway above and below the umbilicus as advocated by others, is that it is sufficiently large for the extraction of the child, and at the same time permits accurate exploration of the pelvic contents, as well as amputation of the uterus should an unexpected necessity for a radical operation arise. Whereas, with the high and mid-umbilical incisions the abdominal wound must be extended downward before the pelvic contents become accessible.

Suture of Uterine Incision

Passing to the method of suturing the uterine incision, it is found that considerable divergence in practice prevailed during the early and later periods of the series. In the early cases the uterine incision was closed with deep, interrupted silk sutures, which passed down to but did not include the decidua. After a while we began to use fine interrupted silk sutures between the deep sutures, in order to secure better approximation of the peritoneal layer; a little later catgut was substituted for silk, and finally, the method was evolved which is now employed. For years we have laid the greatest stress upon the manner in which the uterus is sutured and are well satisfied with the following procedure: As many deep interrupted chromic catgut sutures as necessary are laid. These extend through the bulk of the muscle, but do not include its superficial layer or the peritoneal covering. If, after they are tied and cut, there is any gaping of the muscle between the sutures, a continuous catgut suture brings together the gaping points; and finally, the peritoneum and superficial muscle are brought together with a single continuous catgut suture, which completely buries the knots of the deep sutures and brings the peritoneal edges into the closest approximation.

Our records show that the uterine incision was closed with silk in 20 and with catgut in 106 conservative sections. As with but few exceptions, silk sutures were employed at the same time as evisceration was practised, it is impossible to draw definite conclusions as to whether the employment of the former had any influence upon the course of the puerperium, or whether the comparatively poor results obtained at that time was associated with the evisceration of the organ, and particularly with the selection of patients for operation.

Rupture of Cicatrix in Subsequent Pregnancy

Closely allied with the question of suturing the uterus is the consideration of how the resulting scar will stand the distention incident to subsequent pregnancies. A voluminous literature has accumulated upon the subject, and it is generally believed that the scar yields in from 3 to 5 per cent of subsequent pregnancies, when the uterine contents are extruded into the abdominal cavity with the result that the child is inevitably lost and the mother perishes from infection or hæmorrhage unless promptly operated upon. Of course, this accident depends in part upon the manner in which the uterus was orginally sutured, and particularly whether its healing was complicated by infection. The possibility of its occurrence has been so emphasized that the dictum "once a Cæsarean, always a Cæsarean" has obtained very general acceptance. In our experience, however, the danger of subsequent rupture has proved to be less than is generally believed, and we attribute its relatively infrequent occurrence to the care with which we have closed the uterine incision. Rupture occurred but once in 48 women who had repeated sections in the service, as well as in 12 others who were delivered by the natural passages following a previous section.

An interesting point in this connection is that in many of the repeated sections no trace of the cicatrix of the previous section could be noted on inspecting the unopened uterus at the second or third operation; while in other instances the only evidence of it consisted in adhesions over the anterior surface of the uterus, which apparently involved the old scar. So far as I can recall, with the exception of the actual case of rupture, signs of stretching of the cicatrix were noted in only a single uterus before it was incised at the subsequent section. On the other hand, when we came to study the conditions obtaining in the 21 uteri which were amputated at a final section, we found a varying condition of affairs. In many instances no trace of the cicatrix could be observed; in others the site of the previous incision was marked by a shallow depression upon the inner or outer surface of the uterus; while in a still smaller number a longitudinal depression upon both its outer and inner surfaces marked its site, and had led to a greater thinning of the uterine wall there than elsewhere. Microscopic examination, however, showed that, irrespective of the gross appearance of the cicatrix, no trace of scar tissue remained, and that the muscle fibers crossed the site of the original incision as if it had never been made. I shall not enter into details concerning this subject, for the reason that my assistant, Dr. Thomas O. Gamble, is now engaged in an extended study of all of its phases.

Site of Placental Implantation

Many writers upon Cæsarean section advise that every effort should be made to locate the situation of the placenta in order that the uterine incision may be made in such a location as not to involve it. Although for years we have made it a rule to attempt to locate before operation the situation of the placenta by determining the course of the round ligaments, and still continue to do so as a matter of diagnostic interest, we make no attempt to avoid the placental site, and as a matter of routine incise the uterus in the midline of the lower part of its anterior wall, except in the rare instances in which it is so pendulous that its posterior wall lies in contact with the anterior abdominal wall.

At the same time, figures concerning the location of the placenta may be of interest, and analysis of the notes which were available in 157 operations gives the following results: posterior implantation, 94, anterior implantation, 62, placenta prævia, 1. In other words, anterior implantation was observed in 38 per cent of the cases; and, consequently, if the anterior wall be incised as a matter of routine, the placenta will be involved in two cases out of five.

As has been indicated above, we regard this as a matter of indifference, so that when the placenta lies anteriorly we cut through it and deliver the child through the wound, and the only difference which we have noted is that in such cases there is a profuse preliminary gush of blood, which ceases as soon as the child is extracted and the uterus begins to retract. So far as the convalescence is concerned, our notes show no difference between the cases in which the placenta was incised and those in which it was not.

Use of Pituitary Extract

For many years it was our habit, as soon as the abdominal wall was incised, but before opening the uterus, to inject two barrels of ergotol into the arm or thigh of the patient for the purpose of stimulating the retraction of the uterus and thus preventing bleeding. When pituitary extract came into use we employed it in preference to ergotol. In several instances, however, in which it was necessary to free dense adhesions the action of the drug became manifest before we were ready to incise the uterus, with the result that it became tetanically contracted, and seemed to interfere with the placental function. While this had no serious consequences, we thought it safer to defer employing it until the uterus had been incised, and we then developed the custom of injecting 2 c. c. directly into the uterine substance whenever the uterus did not contract satisfactorily or if the hæmorrhage were excessive. Thus far no deleterious effects have been observed, but in view of the fact that apparently aseptic abscesses sometimes follow the injection of pituitary extract into the muscles of the thigh, it must be assumed that sooner or later such an accident will happen when it is injected into the uterus, when, of course, its consequences may be serious. For this reason we have recently abandoned the practice and now inject the medicament into the muscles of the thigh as a routine procedure as soon as we are ready to incise the uterus.

Condition of Child

It is usually stated that the Cæsarean section child is born in an apnœic condition and sometimes is deeply asphyxiated. This is believed to be due in part to the transmission of the anæsthetic agent through the placental circulation, and in part to the lack of friction which is normally associated with birth through the natural passages. Upon analyzing our histories I was surprised to find that such conditions were encountered less frequently than is ordinarily stated, and from the 145 histories which contained notes concerning the establishment of respiration in the child, we have collected the following figures:

Cried at once	75 cases	(51.7%)
Slightly asphyxiated	60 "	(41.4%)
Deeply asphyxiated	10 "	(6.9%)

In 16 other cases the child was born dead, but in no instance could its death be attributed to the operation, as in all such cases the section was undertaken for the sake of the mother and quite irrespective of the condition of the child. Consequently, it appears permissible to conclude that if the child is alive and in good condition when the operation is begun, it will be born alive, after which its chances for prolonged life will be the same as after normal delivery.

Formation of Adhesions

Another point in connection with the convalescence from Cæsarean section is the occurrence of adhesions between the uterine wound and other structures. In the old days, before aseptic technique had been developed and when the retraction of the uterus was relied upon to check hæmorrhage without the application of sutures, the formation of dense adhesions between the uterus and anterior abdominal wall was the rule. At that time this was so much the case that one expected in subsequent operations to deliver the child through a utero-abdominal fistula without opening the peritoneum.

So far as I can ascertain, no figures are available concerning the incidence of such adhesions following the modern technique, and although it is often possible by examination of the living woman to determine their existence, no accurate statement can be made as to their frequency. On the other hand, when repeated sections are done, definite conclusions can be drawn by noting the condition of affairs at the time of operation. Thus, upon analyzing the 48 such cases in our series we find the following:

	Cases
No note	2
No adhesions	12
Slight adhesions	10
Omental adhesions only	6
Broad adhesions	7
Dense adhesions	11

In other words, adhesions were absent in one-fourth, while broad or dense adhesions were present in one-third of the cases.

It is interesting to attempt to determine what relation, if any, the character of the convalescence bears to their formation, and whether a febrile puerperium favors their development. As in 10 instances the preceding section was done elsewhere, it is impossible to make any statement concerning the convalescence following it; but in 37 cases we have notes concerning the previous convalescence and the accompanying table summarizes the findings:

Convalescence,	Normal	Febrile
No adhesions	4	7
Slight adhesions	3	7
Omental adhesions	2	3
Broad adhesions	3	2
Dense adhesions	2	4
	14	23

In other words, the puerperium was normal in 38 and febrile in 62 per cent of the cases, and adhesions of the various types occurred after each, but were nearly twice as frequent after a febrile convalescence. It is, therefore, apparent that while a febrile puerperium appears to favor their formation, an afebrile puerperium does not necessarily insure their absence.

This being the case, it seems justifiable to infer that the occurrence of adhesions is not always the result of actual infection, but may quite as well be associated with the presence of raw surfaces resulting from defective methods of suturing the uterus, or from other traumatic factors. Such a conclusion is only hypothetical, but a certain degree of probability is lent to it upon considering the differences observed according as the first section was done elsewhere or in our service, as it may be assumed that it is possible that other operators sutured the uterus less carefully than we. Thus, broad or dense adhesions were noted in seven out of the nine

operations done elsewhere, as compared with 11 in the 37 cases done here. In other words marked adhesions developed in 76 and 30 per cent of the two groups respectively.

CORPUS LUTEUM AND SEX OF CHILD

From the time of Hippocrates to Rumley Dawson, many writers have believed that boys are derived from ova originating in the right and girls from those in the left ovary. Since our attention had been directed to this point, we have made it a practice at Cæsarean section to examine the ovaries carefully, and to note whether the corpus luteum was visible, and, if so, in which ovary it was situated. Notes to that effect were made at 99 operations, and upon analyzing them it was found that no corpus luteum was discoverable in one-third of the cases, while it was present in the other two-thirds.

When the corpus luteum was present in the right ovary 23 boys and 13 girls were noted, as compared with 16 boys and 12 girls when it was situated in the left ovary. In other words, in our experience, more boys than girls were obtained, irrespective of whether the corpus luteum was in the right or in the left ovary, and thus convincing evidence is adduced against the correctness of the theory that boys are derived from one and girls from the other ovary.

An interesting point in connection with these figures is that they demonstrate very clearly the fallacy of attempting to draw conclusions from too small a number of cases. For example, in the 64 cases in which the corpus luteum was present and the sex of the child was noted there were 25 girls and 39 boys, a ratio of 100 to 158, as compared with the normal of 100 to 106. With such figures at hand, an incautious person might be tempted to draw various erroneous conclusions, as for example that the existence of contracted pelvis or of some other condition which necessitates Cæsarean section may lead to a predominance of boys. That such a conclusion is unjustifiable, is shown by the fact that when the entire number of sections was considered the ratio of girls to boys was 100 to 107, which closely approaches the normal, and indicates that the former abnormal ratio was entirely accidental and of no particular significance.

CONCLUSIONS

1. This analysis is based upon 183 Cæsarean sections performed upon 145 women up to December 31, 1920.

2. The operations were done in a series of approximately 20,000 deliveries, and comprise 104 single, and 79 repeated sections. The latter were done upon 41 women, 34 of whom had two, and 7 three sections each.

3. Although the number of white and black patients in the service was approximately identical, many more Cæsarean sections were done upon the latter—114 to 69, while 30 to 11 required repeated sections.

4. The following types of operation were done:
 121 typical conservative sections.
 4 extraperitoneal sections.
 1 postmortem section.
 57 Porro sections.

5. The gross mortality was 5.46 per cent, but, upon deducting the cases in which death was not attributable to the operation, the net mortality was 3.45 per cent; or 4.07 per cent in the conservative and 1.82 per cent in the Porro sections.

All deaths, except one from hæmorrhage were due to infection.

6. The mortality was 13 times greater in the first 50 than in the last 133 cases—10 to 0.77 per cent. This remarkable diminution was not due to changes in operative technique, but to the avoidance of ascending infection by operating before the onset of or during the first hours of labor.

7. The conservative section late in labor is always dangerous, even if vaginal examinations have not been made; while the Porro section is relatively safe. The most important means of lowering the mortality of conservative Cæsarean section due to disproportion is by learning to determine before the onset of labor whether operation will be required or not.

8. The Porro operation is relatively safe even in infected or exhausted patients, as the absence of the involuting uterus hinders the spread of infection.

9. Disproportion due to contracted pelvis was the indication for interference in nine-tenths of the black, and in six-tenths of the white patients.

10. The several varieties of rachitic pelvis afforded the predominant indication in the blacks, as compared with the simple flat pelvis in the whites.

11. The most frequent non-pelvic indications were eclampsia and serious cardiac decompensation.

12. Cæsarean section is not the ideal treatment for eclampsia, and is indicated only in the rare instances in which the cervix is rigid and undilated and venesection has not led to improvement.

13. It is likewise only rarely indicated in placenta prævia. We have done but one section in 66 cases, and regard the rubber balloon as the best treatment.

14. Generally speaking the patient should be sterilized at the third section, either by amputating the uterus or by an operation upon the tubes.

15. We make the abdominal incision below the umbilicus, as it permits amputation of the uterus or operations upon the appendages, when necessary, without extending the incision.

16. The uterus should be incised *in situ,* and eviscerated before incision only in the presence of infection. Our experience indicates that in normal cases the latter procedure increases the incidence of infection.

17. The uterine incision should be sutured in layers, and the greatest care taken to insure the closest approximation of the peritoneal margins.

18. The uterine cicatrix ruptured once in 48 women with repeated sections, as well as in 12 deliveries through the natural passages subsequent to section. The frequency of its occurrence is probably exaggerated, so that the dictum "once a Cæsarean always a Cæsarean" is not necessarily correct.

On the other hand, the possibility of rupture must always be faced and constitutes the strongest argument against the unnecessary employment of Cæsarean section for non-pelvic indications.

19. The placenta was inserted upon the anterior wall of the uterus in two out of every five of our cases. Consequently, it is frequently involved in the uterine incision. This has no other significance than a momentary gush of blood.

20. The delivery of an asphyxiated child occurs less frequently than is generally believed. Somewhat over one-half of our children cried immediately after delivery, and only 7 per cent were deeply asphyxiated.

21. Notwithstanding the extraordinary value of pituitary extract in stimulating uterine contraction, pronounced atony with danger of death from hæmorrhage is still to be reckoned with, and necessitated amputation of the uterus in two of our patients.

22. Uterine adhesions were absent in one-quarter of our repeated sections, and were extensive in one-third of them. They are not necessarily the result of infection, as the puerperium was normal in 36 per cent of the cases in which they developed. In many instances they appear to be associated with imperfect approximation of the uterine wound or with other traumatic factors.

23. The old superstition that boys originate from the right and girls from the left ovary can be definitely discarded. In two-thirds of our patients the corpus luteum persisted until the end of pregnancy, and its location bore no relation to the sex of the child.

24. Finally it should be remembered that Cæsarean section is not devoid of danger, and is relatively safe only when done under appropriate conditions before the onset or during the first hours of labor.

As the uterine cicatrix constitutes a *locus minoris resistentiæ* in subsequent pregnancies Cæsarean section for other than pelvic indications should be performed only when absolutely necessary.

It is my conviction that the operation is being abused throughout the country, and if accurate statistics as to its results were available that it would be found to be accountable for many unnecessary maternal deaths.

It should be recognized that, although it is frequently the easiest manner of delivering the patient in the presence of various abnormalities, it is not always the safest, and that ideal results are obtained in only a few clinics.

MODERN METHODS IN HANDLING HOSPITAL STATISTICS[1]

By Raymond Pearl,
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I. Introduction

To an ever-increasing degree modern science is becoming quantitative in its methods of thought and activity. The history of science from the beginning shows that the earliest development of any discipline is purely qualitative and that only as it emerges from this state and passes over into the quantitative phase, in greater or less degree, does it begin to take an assured place in the hierarchy of the established sciences. Recent examples of this change from a qualitative point of view are found in psychology and sociology. With the development of knowledge and of an appropriate technique eventually any natural phenomenon which can be observed can also be quantitatively measured. The entire history of medicine shows that there has been almost from the first an earnest desire and effort, on the part of some of its leaders, to develop quantitative modes of thought and methods of work. The large measure of progress which has been made in this direction is sufficiently evidenced by the number of items of diagnostic and clinical significance which are measured and recorded in quantitative terms.

The analytical treatment of the quantitative data of medicine has developed far more slowly than the appreciation and collecting of the data themselves. This fact is neither surprising nor peculiar to medicine. Only in the last decade of the nineteenth century with the pioneer work of Galton, Weldon and Pearson, did there begin the application of really adequate statistical methods to any sort of biological problem. Since that time the development has been very rapid in many of the fields of general biology. In medicine there have been differences of opinion, as would be expected, between leaders, as to the significance and importance of a really scientific statistical calculus in the field of medicine. The reactionary and the progressive viewpoints in this regard cannot be better set forth than in the following quotations from two clinicians of the foremost rank. In 1912 Sir Almroth Wright, in discussing the statistical method, said:[2]

> These general considerations have prepared the way for bringing forward the suggestion that the ordeal of minutely accurate quantitative statement which is always floating before the vision of the statistician should in the field of clinical medicine be frankly abandoned. This would mean recognizing that it is, in medicine, impossible by the method of cumulative experiments either (a) to detect minute differences, or (b) to arrive in any case at an accurate quantitative conclusion. . . . . Where there is a conflict of opinions between observers the proper course to pursue is to appraise the relative weight of the authorities who are ranged over against each other.[3]

[1] Papers from the Statistical Department of The Johns Hopkins Hospital, No. 1.

[2] Wright, A., and others. Observations on the pharmaco-therapy of pneumococcus infection. Lancet, 1912, (2), pp. 1701 and 1704.

[3] It should be pointed out that Sir Almroth Wright's strictures upon the statistical method have been dealt with by Greenwood (Lancet, 1913, Jan. 18) in a wholly adequate, and, for such persons as are capable of logical thought, final manner.

In 1920, Lawrason Brown said:[4]

None of you will contradict me when I say that statistics are very dry, but some of you may dispute me when I say that only by statistics does the world, lay or medical, advance. Consider what knowledge is and you will see how inseparable it is from statistics. Medicine is no exact science, and diagnosis rests largely upon the law of probability which in turn is statistical. All scientific experiments are statistical arguments in favor of or in opposition to certain inductions or deductions. Further, statistics lend the authority that is necessary for their acceptance.

The trouble in medicine does not lie with the statistical method but with the medical men who do not know how to use it. I regret to state that I belong to this class and have felt keenly that in medical school I did not have an opportunity to attend a course on medical statistics. The day will come, gentlemen, when such courses will be given, when the law of probability will help in diagnosis, when the coefficient or correlation, now explained by most authorities in such terms that in a few minutes my idea of my relation to my surroundings has become totally insufficient—when, I say, all these things will be understood by the medical graduate. At that time medical men will cease to do such foolish things with statistics as to try to add cabbages and cows, or, what is nearly as bad, to try to solve problems in heredity by finding how many parents had the disease from which the offspring suffers without due respect to many other very important and possibly contradictory details. What would you think of a bookkeeper who after years of personal experience would gather up the bills in the cash drawer and go to the bank with the statement that his personal experience led him to believe that the roll of bills amounts to $1000. The receiving teller would quickly apply the statistical method and few would venture to side with the bookkeeper, no matter how large his experience had been.

Do not misunderstand me. This is not an argument in favor of dry statistical articles which we all prefer to avoid reading. But if I can make you see how important it is for us to cease using the pet phrase "my personal experience" except when we have sufficient data to support it, I shall have accomplished what I had hoped for.

General experience with other branches of science would make it seem reasonable that the following propositions are true, and should be emphasized in the teaching of medical students:

1. That there is no inherent reason why medicine in every one of its phases should not ultimately become in respect of its methods an *exact* science, in the same sense that physics, chemistry, or astronomy are to-day exact sciences.

2. That this goal will be reached in exact ratio to the extent to which quantitative methods of thought and action are made an integral part of work of every sort of medicine.

3. That no number or figure can be said to have any final scientific validity or meaning until we know its probable error, the "probable error" being the measure of the extent to which the number will vary in its value as the result of chance alone.

For at least three centuries it has been the custom of hospital authorities to prepare and publish routine statistics regarding the activities of their institutions. These statistics usually comprise only the following general sorts of items: Number of admissions, nature of ailment, number of treatments, number discharged either dead or alive, improved or unimproved, and other similar material.

It is interesting to speculate as to why such statistics are published. I believe the clue is found in the history of the matter. Hospitals began as public or private charities, to the support of which financial contributions were solicited. The evolution of human nature having proceeded no farther than it had, it was felt necessary to furnish at frequent intervals documentary evidence of the moral rectitude of those persons responsible for the disbursement of the donated funds. The best possible evidence that someone has not absconded, or misused the institution's resources is statistical proof that the hospital has been performing its proper function of admitting, treating, and discharging patients. Beyond this essentially moral purpose it is difficult to discover that the type of purely routine hospital statistics now referred to has, will, or can serve any useful purpose. I am aware that at various times statisticians have presented memoirs and reports upon hospital statistics of this sort, but in general such reports appear to me essentially to have done only one or the other of two things: Either (1) they have shown that the treatment of disease in hospitals is more effective now than it was at some earlier period; or (2) they have attempted to deduce conclusions as to the incidence in the population of different forms of disease. Regarding the first of these points it may fairly be said to fall under the sort of activity which William James called "the painstaking delineation of the obvious," a practice which has deservedly brought statistical science into disrepute upon more than one occasion. On the second point it need only be said that to attempt any deductions from hospital records as to the normal incidence in the population of either morbidity or mortality is an obviously fallacious procedure, since the hospital population is a highly adversely selected sample of the general population. This fact has, of course, been appreciated widely. There have been a few scientifically sound and valuable contributions to knowledge based upon purely routine hospital statistics, but the number is meager.

It is obvious, in the nature of the case, that a large general hospital comes by way of a literally enormous number of facts about disease and its treatment, many of which facts are either now expressed in numerical form, or could be easily so expressed. In a broad sense, disease is a biological problem, to the solution of which every case can be made to contribute some mite of data or knowledge. The case history is the tangible form which the record of these individual facts takes. When, however, a hospital has been running any significant length of time, the number of these individual case histories becomes so vast that nothing can be done with the wealth of data which they contain except by the application of the statistical method. Now the modern statistical calculus is a highly intricate and complex branch of higher mathematics. It is unreasonable to suppose that the same person is likely to be, except in the rarest instances—of which a brilliant example is Dr. Major Greenwood of the British Ministry of Health—an expert in both medicine and statistical mathematics. Yet the investigator in medicine, by force of the very

[4] Brown, Lawrason. American Review of Tuberculosis, September, 1920. Volume IV.

nature of his data, is compelled to use the statistical method in analyzing and presenting his results. The result is sometimes a little sad if judged by the same canons of scientific method as those by which the published work of a physicist, for example, is judged. But if we leave aside these deplorable cases, happily few in number, of wrong conclusions from data, and consider only those results which may be regarded as correct so far as they go, the trained statistician is able to see in a great many cases how, by proper mathematical treatment of the existing data, it would have been possible to get a much deeper insight into, and much more useful knowledge about, the problem than the statistically untrained medical man was able to get.

The case is plainly one calling for the cooperative endeavors of specialists in distinct fields. Since a modern hospital moves and has its being in the idea and practice of cooperation between various medical and surgical specialists it would seem a singularly happy environment in which to develop cooperation, having as its ultimate object the best use for the advancement of the science of medicine of the quantitative data which its records furnish. This means that the statistical records of a modern hospital should be supervised by and their investigational analysis undertaken in cooperation with an expert statistician.

II. Some General Principles to Be Observed in Developing Statistical Work in a Hospital

1. Since one cannot get out of any machinery of statistical treatment anything intrinsically different from what went in, it follows that accuracy and completeness of the initial routine statistical data collected in a hospital are matters of prime importance, in which everyone interested in the work of the hospital is also *per se* interested, and needs only to be shown where any improvement can be practically made, in order to give his cooperation to the attainment of that improvement.

2. In any old and well-established hospital the existing system of case record keeping is sure to be so intricate and has so many ramifications, that changes made in it, if any, should be very gradual, and only with the complete and hearty approval of everyone involved. At the same time, however, it should be recognized that there have developed in recent years great advances in the art or science (as one pleases) of efficient record keeping. Some of the older hospitals in this country operate with a general record system which unquestionably violates nearly every one of the best established canons of modern office practice, and which would not be tolerated for a moment by any equally large, properly organized and managed, business concern.

3. The statistical method is a *technique,* a means of getting light on complex problems and not an end in itself, and therefore it is essential that before it be applied there be a clear definition of the problem to be attacked, whether biological, medical, pathological, surgical, or other. Unless there is a real problem, with real meaning, to be solved, the application of the statistical method is as futile and sterile as would be the application of any other technique.

4. The development of research work along statistical lines in a hospital should be just as rapid, and only as rapid, as the results obtained demonstrate the value to the advancement of medical science in general of this type of work.

5. Since the statistical technique is a highly complex one, requiring expert knowledge for its correct and most useful application, an expert statistician should certainly be a member of the staff of any hospital attempting to do any research work, and should be called into consultation in regard to the methods and form of research communications based upon the statistical data of the hospital, in order that duplication of effort, and the commission of egregious errors in drawing conclusions from quantitative data may be avoided. Furthermore, for the benefit of medical science in general, and in the interest of better administrative and professional operation of the hospital itself, every large hospital should have at least one properly trained person to attend to the assembling and tabulation by adequate modern methods the routine statistics of the hospital.

III. Functions of a Statistical Department in a Hospital

The statistical department, in accordance with the above outlined general principles, should perform the following functions, either directly or in a supervising and directing capacity:

1. *Assembling and Tabulating Routine Statistical Data.*—The case history room is the brain of a hospital. There are finally assembled all the records that exist of the medical (using the term in the broadest sense) activities of the institution. To make histories available for future reference for whatever purpose, the data which they contain must be classified and indexed in some manner or other. Further, if any comprehensive view is to be had of the medical activities of the hospital, these data must from time to time be tabulated in a great variety of ways to meet the variety of needs and interests certain to be reflected in the intellectual activities of the staff of any large teaching hospital. Now, as presently will be shown in detail, this indexing and tabulating can all best be done by modern "mechanical tabulation." If it is so done, an incalculable, but certainly very great, addition will be made to the efficiency of the hospital's work. There is no more depressing sight to the statistician, familiar with efficient methods of handling large masses of data, than to see a poor interne struggling with the index and bound volumes of case histories, painfully and slowly assembling on great sheets of paper all the records the hospital has about some particular diseased condition. Months of labor may be, and all too often are, spent in the mere *assembling* of a small collection of data from the case histories, which a properly organized statistical department, with facilities for mechanically indexing, sorting and assembling the data, could easily furnish in a half day. So, then, the first duty of a hospital statistical department should be the transferring to punched cards for purposes of indexing, assembling, and tabulating, of the basic routine facts from all case histories.

2. *Organization of Special Departmental Records.*—The routine statistical records discussed in the preceding section form only the skeleton of the whole statistical structure which one can see developing in hospitals. Different departments and services will want, as a routine matter, much more elaborate analysis and indexing of the facts of their own cases than would be either proper or possible as a basic uniform procedure for the hospital in general. An important function of the statistical department will be to aid in the development, proper handling, and mechanical tabulation of these special and detailed but still routine statistics. An illustration will be given further on in the paper of the possibilities of this type of statistical development, in connection with the prostatectomy histories of the Brady Urological Institute of this hospital.

3. *Consultant Service.*—A very large percentage, indeed nearly all, published papers based upon hospital or other medical records, contain a greater or smaller amount of statistical data. In many cases these papers are written by men who know practically nothing about the proper treatment of statistical data in such a way, on the one hand, as to lead to correct conclusions, properly fortified with probable error measures of reliability, or on the other hand, to get the maximum of useful information out of the data themselves. As has already been pointed out this difficulty is inherent in the nature of the case, medical training being as it now is and as it is likely to be for some time to come. It implies no criticism of anyone, but is merely one expression of the patent fact of the specialization which exists in all fields of modern science. The best solution of the difficulty would appear to be to build up such a spirit of cooperation between the statistical and other services of a hospital, that the former would regularly be called upon, in a consulting capacity, to aid in the analysis of the purely statistical phases of the research work done in the institution. This would involve a wide variety of service, ranging all the way from simple advice as to the best form in which to present statistical tables or charts, to long computations to bring out in the best way the significance of the results. In this field the highest intellectual equipment and expert knowledge of the statistician will be called for. But there can be no doubt that in any hospital where such a plan of cooperation is effectively utilized, the quality of the research output will be immeasurably raised. It must, of course, be understood that in this field, as in most others, cooperation is a delicate flower, whose growth cannot safely be forced. One proceeds most rapidly by going slowly. Our experience in The Johns Hopkins Hospital indicates, however, that as soon as the underlying viewpoint and significance of this sort of consulting service is well understood by the staff, the requests for it come along quite as rapidly as a small statistical staff finds desirable.

4. *Research.*—The properly organized hospital statistical department will have as its head an investigator, who will see in the wealth of material which exists in the records possibilities, undreamed of by the non-statistical clinician, of throwing light on obscure problems of medicine and surgery. The carrying on of special biometric investigations, based upon hospital records, is one of the highest functions which a statistical department can perform.

IV. The Commonest Defects in Hospital Records

The fundamental and basic element in a hospital's records is the individual case history. Upon it depends any and all useful information, whether statistical or otherwise in character, which may be wanted for any purpose whatever. It is, therefore, of the highest importance that hospital histories conform to the best standards of scientific record making, on the one hand, and of modern office practice on the other hand. There are relatively few hospitals where the highest standards in either of these respects are even approximated.

From the standpoint of scientific routine record taking, case histories are most glaringly defective in what they *fail* to record about the patient. It is by no means impossible to find case histories that fail to record the sex of the patient, while any indication of what *kind* of person the patient was, in the common sense of the word, whether fat or lean, white or colored, rich or poor, young or old, etc., is all too frequently kept a deep secret from any subsequent reader of the history. Again, even in the special medical portions of the history the writer forgets, with almost unbelievable frequency, to make any record of highly important facts.

The root of such difficulty apparently lies in the method by which case histories are written. The general scheme or outline which a history is to follow resides, far too often, in the head of the history writer, and there only. And heads, especially of human beings, do vary so! The remedy is patent. Any hospital or service that desires to put its clinical records on the case record form for gall-stone cases, for example, there have printed a series of *standard* history forms, which will cover not merely general routine facts common to all diseased conditions, but special forms as well, for at least all of the more frequently occurring conditions. These blank forms will contain definitely indicated spaces in which some statement of fact *absolutely must be recorded in every single case.* If on the most scientific basis will as a first step draw up and is printed the question, " Did this patient ever have typhoid? " or the equivalent of this question, and if furthermore every worker in the service clearly understands that any history for which he is responsible that comes into the history department, with any blank spaces in its standardized portion, will not be accepted for filing, but will be forthwith returned to him for completion, future students will not be under necessity of having a " No information " column in their statistical tabulations relative to this point.

One realizes perfectly that any suggestion in the direction of standardizing case history writing, by the process of putting into operation methods which have been found sound and useful in other branches of science and in modern business, will at once be scornfully or even derisively received by some. It will be argued that any such process tends to cramp their individuality. This argument is perfectly valid. It will inordinately cramp such portion of their individuality as finds

its expression in carelessness, inaccuracy, forgetfulness, and inattentive observation. In so far as it is desirable to foster and preserve these intellectual qualities, and embalm their results in the permanent archives of a hospital, clinicians and surgeons should be encouraged to go on writing histories in the old way.

Furthermore, the argument will be made that the writer, a layman, has no competency or right to discuss at all the manner in which case histories are written, because being a layman he can possibly know nothing about the highly esoteric art of medicine. But here a little clear thinking is needed. The science and art of making accurate, comprehensive, and essentially complete records of natural phenomena is not exclusively nor even particularly a branch of the science or art of medicine. It is much broader and more basic and is, in every one of its logical and epistemological principles, common to all sciences. To these principles of scientific record making many persons, including the writer, have devoted many years of study and thought. And it is just precisely *that* field, not medicine, that we are talking about when we are discussing the method of writing case histories.

It is, of course, to be understood that no blank form, however carefully it may be devised, can ever suffice for the recording of the *whole* history. There must be some portions written or dictated with entire freedom from Procrustean rigidities. The reason why this is so is plain. One of the chief characteristics of living things, whether men or mice, is that they vary individually. But formal blanks do not vary. An invariable phenomenon cannot fit a variable one. But this is no valid argument against having certain essential parts of the history recorded in standardized form. There are certain facts that everyone will agree ought to form a part of every history case which is to be permanently preserved. It is that class of facts which should be recorded upon standardized formalized sheet or sheets incorporated into each history. Then *in addition* the clinician may write or dictate as much more as he likes, in an entirely free untrammeled style. The formalized portion merely serves as the schema of the whole, to make sure that no point of importance for future students is left out, because forgotten, in the greater interest of other more immediately exciting features of the case.

Turning to the question of the way case histories are handled after they are written, which is essentially a matter solely of business or office management and not of medicine, there are two glaring defects in the common practice. These relate, first, to the fixation of responsibility for the recording of each item in the history, and, second, to the filing of the completed histories. From every point of view, whether of administration, research or other, it is of the highest importance that future students of a hospital's records should know who is responsible for statements appearing in a history. How often has one seen and heard long and inconclusive debates as to what interpretation was to be put upon some statement in a history as to a clinical finding? The decision all depended upon who originally was responsible for the statement. If it were the considered verdict of the wise and experienced old professor it was one thing; if it were the snap judgment of the latest interne it was quite another. All this difficulty can be removed by inaugurating and practicing the principle that every sheet of a history shall bear upon its face the names of the person or persons responsible for what appears upon that page. Perhaps a word of caution needs to be added lest there should be some misunderstanding. Fixation of responsibility is not to be construed as an excuse for any weakening of the rigid canons of extreme objectivity in history or protocol writing, now generally taught in all first-class medical schools.

The purpose of *filing* case histories is two-fold: first, to preserve them, and, second, to do it in such a way as to make them most readily accessible to anyone who may in the future want to consult them. There can be no question that this latter purpose will best be served by the so-called "unit system" of case histories, in which the hospital's complete record about any one individual forms one separate and distinct volume. The advantages of this method of preserving histories over the far more common system of binding them up in great volumes in numerical or temporal sequence, are so obvious as not to need detailed exposition. Such a method of handling the completed records is really essential to their most efficient utilization, whether for statistical, investigational or any other purposes.

V. The Organization of the Routine Statistical Records of a Hospital

There are certain items of information which ought to be and generally are intended to be included in every case history. Some of these routine items are:

1. Case number.
2. Service number.
3. The patient's name.
4. Diagnosis.
5. Sex.
6. Social status (single, married, widowed, divorced).
7. Age.
8. Occupation.
9. Body weight.
10. Stature.
11. Race.
12. Birthplace.
13. Service under which patient was treated.
14. Date of admission to hospital.
15. Duration of stay in hospital.
16. Time from onset of diagnosed condition to admission to hospital.
17. Condition at admission.
18. General health of patient prior to present illness.
19. Whether there is any family history of the diagnosed disease.
20. Whether a first entry or a readmission.
21. Whether a free, a paying, or a part-paying case.
22. Condition at discharge.
23. Whether or not an autopsy was performed.
24. Autopsy number, if any.
25. Nature of treatment.
26. Complicating pathological conditions, additional to the one diagnosed.

In an ideal system of handling hospital records each history should be cross-indexed under each one of the following items

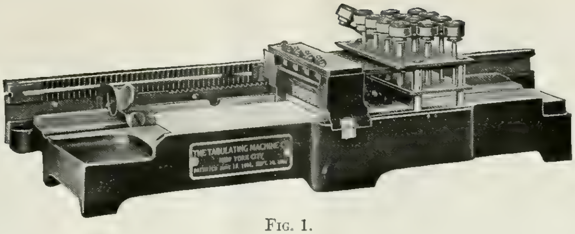


Fig. 1.

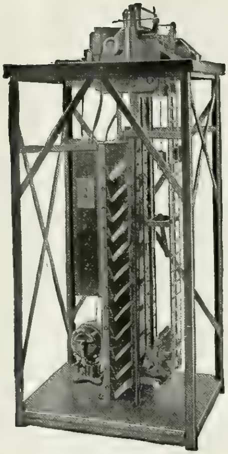


Fig. 2.

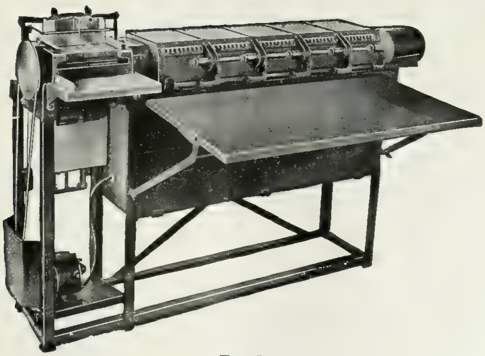


Fig. 3.

in the above list at least: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 21, 22, 23, 24, 25. Of course, nothing like such complete cross-indexing as this is even attempted, not to say accomplished.

There is only one method now known, whereby in a practical way such an amount of cross-indexing can possibly be accomplished. That method is to handle the routine information by the modern system of mechanical tabulating and indexing. On this system the original records are transferred, by means of a machine called a "key punch" (*Cf.* Fig. 1)[5] to cards, the record on the card appearing as a series of punched holes. Then, by means of another machine, known as a "sorter" (*Cf.* Fig. 2), the punched cards can be mechanically sorted, at a rate of 250 cards per minute, into any desired arrangement relative to any character or item of information recorded upon the cards. Let us suppose, for example, that someone wishes to assemble for study all the cases of lobar pneumonia which have been treated in the hospital. Suppose the diagnostic code number for lobar pneumonia is 102. One has then only to run the cards through the sorter relative to the field designated "diagnosis" and pick out, after the cards have been mechanically arranged in numerical order, all those bearing the punched number 102 in the diagnosis field. These 102's will all be together in one bundle, and they will be all the lobar pneumonia cases in the hospital's records. Each card will bear the case number, from which, of course, the original histories can be consulted if one desires. If one particularly wishes to study the lobar pneumonia of negroes, he need only take his bundle of "diagnosis 102" cards, run through the sorter again relative to "race" and he will in a few moments have all the cases of this disease in negroes separated out by themselves. Suppose he is further only interested in lobar pneumonia in negro children under 5 years of age, say. He need only take his bundle of negro lobar pneumonia cases and put them through the sorter again, retaining this time only those falling into ages under 5. He gets his results at the rate of 250 a minute. Compare this with the laborious process that would be involved in assembling by hand from an ordinary card catalogue of hospital case records the case history numbers of *all* the cases of lobar pneumonia in negro children under 5 ever treated in the hospital. The comparison is as of hours with weeks or even months, if the histories be numerous.

Again, suppose that a complete group of like case histories has been assembled by painfully laborious hand processes, and one wishes then to make a statistical tabulation of the facts they contain. Weeks or months may easily be, and often are, spent upon the process. But if the records are upon punched cards, the pertinent cards, which have been mechanically assembled, need only be run again through another machine, known as a "tabulator" (*Cf.* Fig. 3) and the results relative to any desired category of information will be mechanically counted and tabulated, with great rapidity and absolute accuracy, and the columns of figures will at the same time be added.

Examples of the usefulness of this method of handling a hospital's statistics could be multipled indefinitely. But instead of further considering hypothetical cases, let us proceed specifically to the concrete problem of the organization of card forms for the routine statistics of a hospital.

Figures 4 and 5 show the necessary card forms.

A detailed explanation of these forms and the manner in which they will operate is necessary.

A. THE PRIMARY CARD

Taking first the primary card form it may be said that this will presumably be printed upon manila stock. Each group of numbered columns lying between vertical rules is technically known as a "field" of the card.

Across the top of the primary card is written, or typewritten, (a) the full name of the patient, (b) a letter or number designating the service—whether medical, surgical, obstetrical, etc.—in which the patient was admitted; and (c) the number of the case in that service, on the assumption that in addition to the general hospital serial number of each case there is also a special indentifying service number. If a particular service does not specially number its cases, this space will be left blank.

1. The first field is a six-column one and in it is punched the general serial number of the case history. This number identifies the history and the card, and enables one to pass directly from the card to the original history. If a case, for example, is number 12,347 in the hospital's series this field will be punched 012,347. A six-column field permits the separate serial numbering of 999,999 cases. When this number is passed, presumably the cards for the second million would be printed upon stock of another color.

2. The second field of five columns records the diagnosis of the patient's chief or primary ailment. This result is attained by the use of a code of diseases, each pathological condition it is desired to distinguish being given a separate number. A five-column field permits of 100,000 different discriminatory pathological statements. It is to be understood clearly that in this field on the primary card is recorded only what the case history states to be the primary or fundamental pathological condition which the patient presents. The question of the recording of associated and complicating conditions is dealt with below (p. 190). In preparing the nosological code the best advice of the clinicians, surgeons, etc., will, of course, in all cases be taken.[6] The field is made larger on

[5] The most generally useful and flexible system of mechanical tabulation now available is that known as the Hollerith system, from its inventor, Mr. Herman Hollerith. The machines of that system are the ones illustrated in this paper. Further information about these machines may be obtained from the manufacturers, The Tabulating Machine Co., 50 Broad St., New York City. It may be of interest to medical readers to know that a distinguished medical man, the late Dr. John S. Billings, had a great deal to do with the initiation and early development of this invention. He was a close friend and adviser of Mr. Hollerith all through the early stages.

[6] A disease code for use in mechanical tabulation has been very carefully worked out in the Surgeon-General's Office by Major Albert H. Love and his associates.

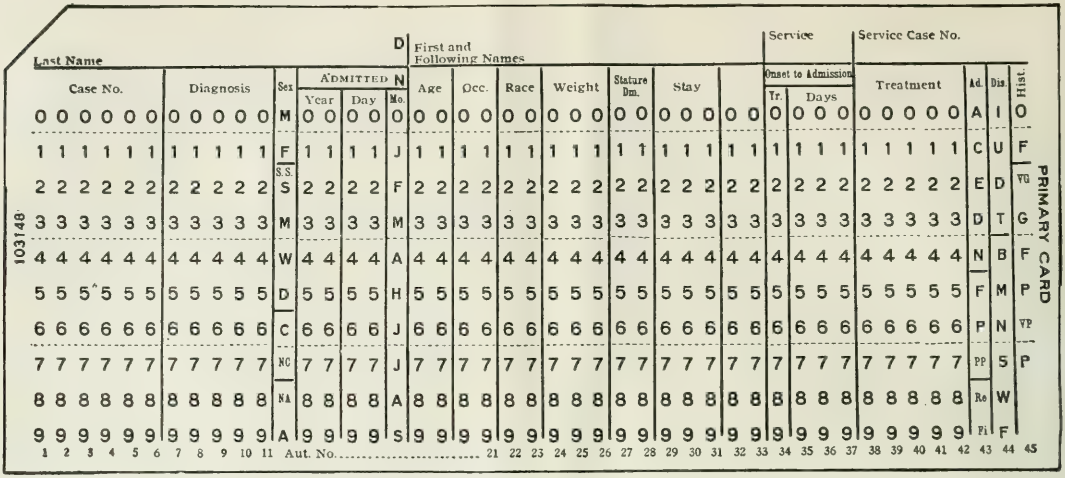


FIG. 4.—First or face card form for mechanical tabulation and indexing routine hospital statistics.

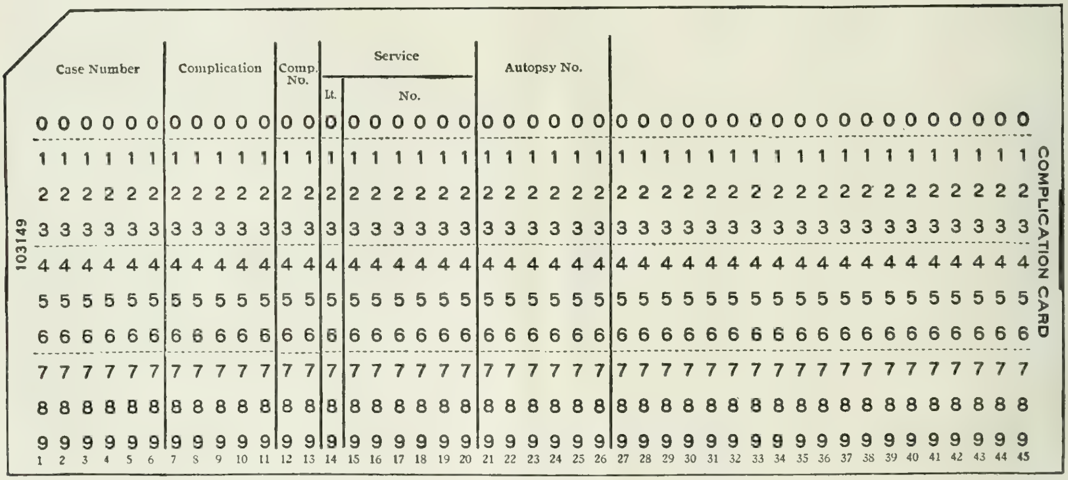


FIG. 5.—Second or supplemental card form for routine hospital statistics.

the card than there is any present need for, to allow for development of the subject and consequent changes in viewpoint.

3. The third field of one column is a "split field" so-called, and records the following information:

(a) Sex of the patient, male (M) or female (F).

(b) Social status, whether single (S), married (M), widowed (W), or divorced (D).

(c) Whether (C) or not (N.C) there were complicating pathological conditions in this case besides the primarily diagnosed condition given in the second field. The presence of this information makes it possible by a single run of the cards through the sorter to separate the uncomplicated cases of a particular disorder from the complicated ones.

(d) Whether (A) or not (N.A) there was an autopsy made in this case. At the bottom of the card is written under this field, in the event that an autopsy was made, its serial number.

4. In the fourth field of five columns is punched the year, month, and day of admission of this patient to the hospital.

5. In the fifth field is punched the patient's age in years.

6. In the sixth field is punched, according to a code, the patient's occupation.

7. In the seventh field is recorded the patient's race, according to the ethnological code used by the U. S. Bureau of Immi-

gration. This information as to race necessarily also covers color.

8. The eighth field of three columns records the weight of the patient on admission, in kilograms (or, of course, if one prefers, in pounds). The most progressive of modern hospitals record weight on admission as a routine procedure.

9. In the ninth field of two columns the stature is punched in decimeters (as close as will ever be used in statistical groupings) or inches, if one prefers as a routine to use common rather than metric measures. The stature in centimeters may be written at the top if the field of the more exact record is desired.

10. The tenth field of three columns records the duration of the patient's stay in the hospital in days.

11. In the eleventh field of three columns is punched the designating letter (by code) or number of the service to which the patient is admitted for treatment, whether medical, surgical, pediatric, etc. The purpose of this is to enable the ready assembling, for any desired purpose, of all the cases in a particular service.

12. In the twelfth, five-column field, is recorded the duration of time in years and days as stated in the history, between the first onset or appearance of the diagnosed condition and the admission of the patient to the hospital for treatment.

13. The thirteenth field of five columns is a very important one. It records, according to a code which can be made as elaborate and detailed as the clinicians and statistician in consultation determine to be desirable, the nature of the treatment given in the hospital to this particular case. Five columns permit of 99,999 separate discriminatory items to be recorded in this field. Suppose, for example, one wishes to study the pneumonia cases in which digitalis was administered, in comparison with those in which this therapeutic measure was not employed. To pick out by hand from all the pneumonia cases the material according to this arrangement would involve an amount of labor which would deter the most enthusiastic young interne. But, mechanically, through the medium of this field, it can be very easily and quickly accomplished.

14. The fourteenth is a split single column field. It records the following information: (a) The nature of the case upon admission, whether an acute illness (A), a chronic (C), an emergency or accident case (E), a case admitted for purposes of diagnosis (D), or a normal person (N), as for example a normal pregnant woman admitted to the obstetrical service for delivery. (b) The financial arrangements of the patient with the hospital, whether a free patient (F), paying (P), or partly paying (P.P).[7] (c) Whether this case represents the first admission of the patient to the hospital, or whether it is a readmission.

[7] In some hospitals, of course, this information under item 14b would not be pertinent and could be omitted or replaced by something else.

15. The fifteenth field is also a single column split, and records:

(a) The condition at discharge, whether improved (I), unimproved (U), dead (D), or transferred to some other service or hospital (T).

(b) The location of the patient's residence, whether in Baltimore (B), or in Maryland outside of Baltimore (M), or in the Atlantic seaboard states north of Maryland (Pennsylvania, Delaware, New Jersey, New York and the New England States) (N), or in the Atlantic seaboard states south of Maryland (District of Columbia, Virginia, the Carolinas, Georgia, Florida) (S), or in some other part of the United States not specified above (W), or in a foreign country.[8]

16. The sixteenth and last field on the card is again a single column split field. It records the following information:

(a) Whether or not (F and O) there is any statement in the written history as to family history of any particular disease in the patient's family. This information enables one interested in the influence of heredity on disease to pick out quickly the cases likely to be of any value to him.

(b) The general health of the patient prior to the present illness, as recorded in the written history. This information is punched according to the following or similar code:

Very good...............	never ill.
Good.....................	minor ailments only.
Fair......................	average amount of sickness.
Poor......................	frequently ill.
Very poor...............	an invalid throughout life.

(c) Whether this card is a primary card. This is a technical point of interest only in connection with the filing of the punched cards.

This completes the description of the primary card. It records 31 different kinds or items of information.

B. The Secondary Card

The basic purpose of the secondary card shown in facsimile in Fig. 5 is to take care of the complicating diseases. An illustration from an actual case history will make the point clear.

A patient, X, was admitted to the hospital under the primary diagnosis of hyperthyroidism and adenoma of the thyroid. A double lobectomy was done. A post-operative broncho-pneumonia developed, and the patient died 14 days after the operation. At autopsy besides the broncho-pneumonia there were found clear evidences of (1) a cerebral embolus with softening, (2) chronic and acute verrucose mitral endocarditis, (3) multiple myomata of the uterus, (4) cystitis, and (5) fibrous pleurisy.

Now the primary card discussed in the preceding section of this paper would carry in the "Diagnosis" field only the

[8] The 15b field is obviously drawn up solely for The Johns Hopkins Hospital and would require modification for any other hospital. Some institutions may not desire statistical information as to place of residence, in which event this portion of the card may be used for recording something else.

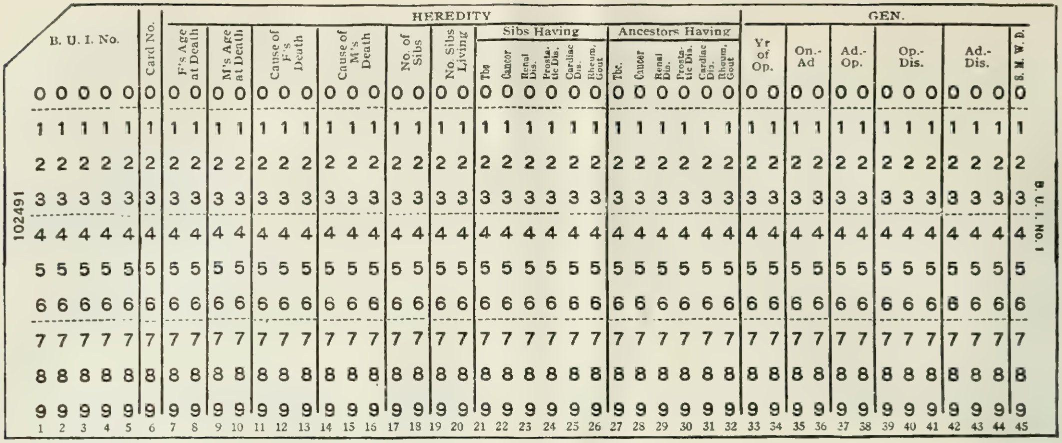


FIG. 6.

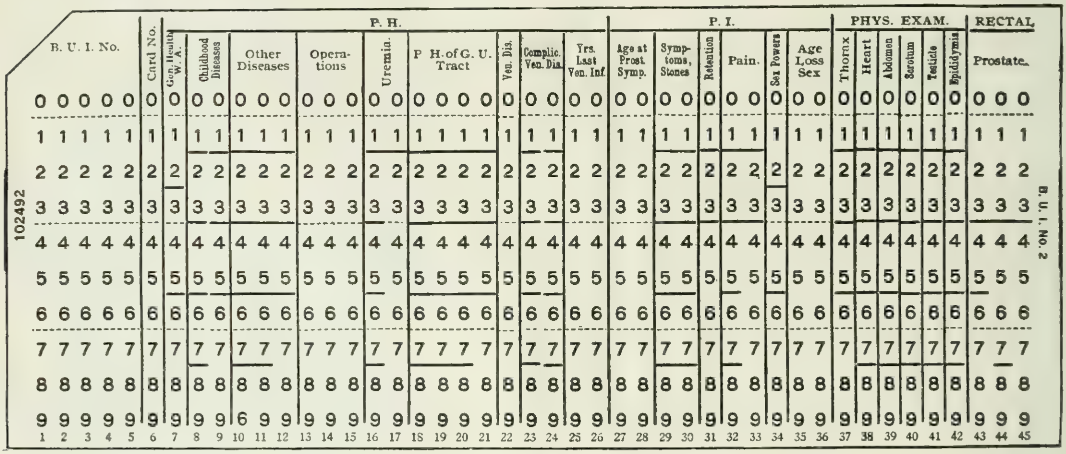


FIG. 7.

adenoma of the thyroid. Yet clearly for any adequate statistical records there must be included some account of the other complicating conditions disclosed by the history. This is done by punching as many of the secondary cards, shown in Fig. 5, as there are separate and distinct complications (that is, in the present case, one secondary card for broncho-pneumonia, one for cerebral embolus, one for endocarditis, one for myomata of the uterus, one for cystitis, and one for pleurisy). Each secondary card carries the same case number in the first field as the associated primary card, and will therefore automatically file with it. The "Complication" field on the secondary card registers with the "Diagnosis" field on the primary card. Therefore, when all the cards, both primary and secondary, are run through the sorting machine relative to this field, all identical diseased conditions, whether primary or complicating, will be brought together. Then by a second sorting of the cards the cases in which any particular disease, say broncho-pneumonia, was the occasion of admission to the hospital, can be separated from those cases in which this disease was a secondary complication.

The remaining fields on the secondary card are used to record additional information for which there was not space on the primary card. These include (1) the ordinal number of the complication as recorded in the history, (2) the service number which was written but not punched on the primary card, and (3) autopsy number, which also was written but not punched on the primary card.

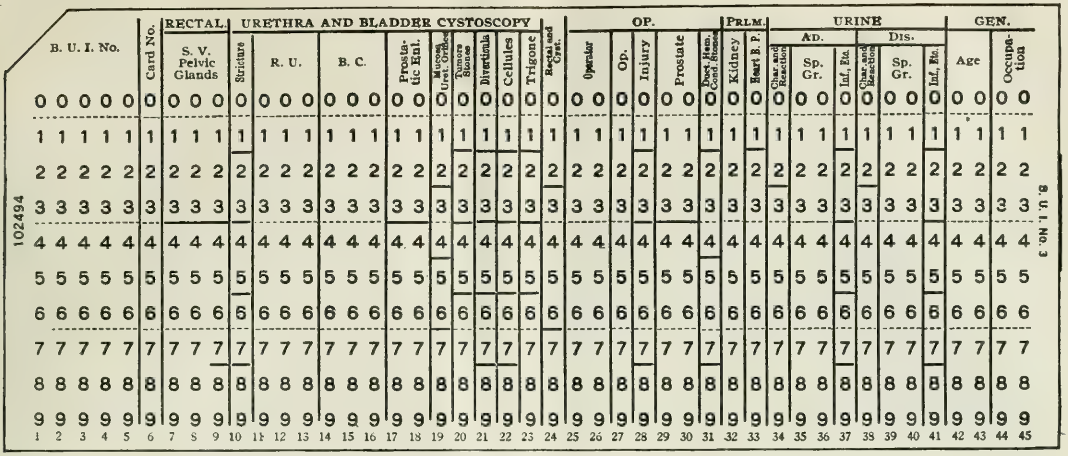


Fig. 8.

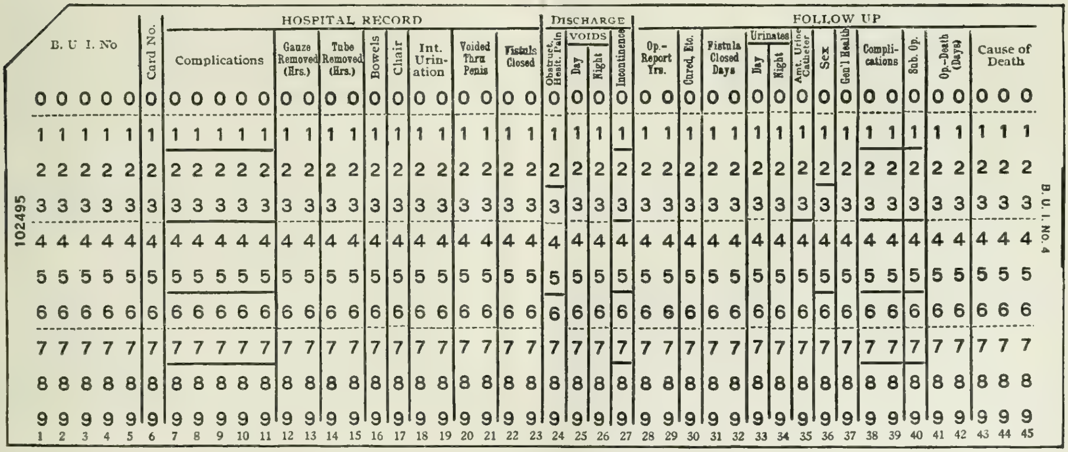
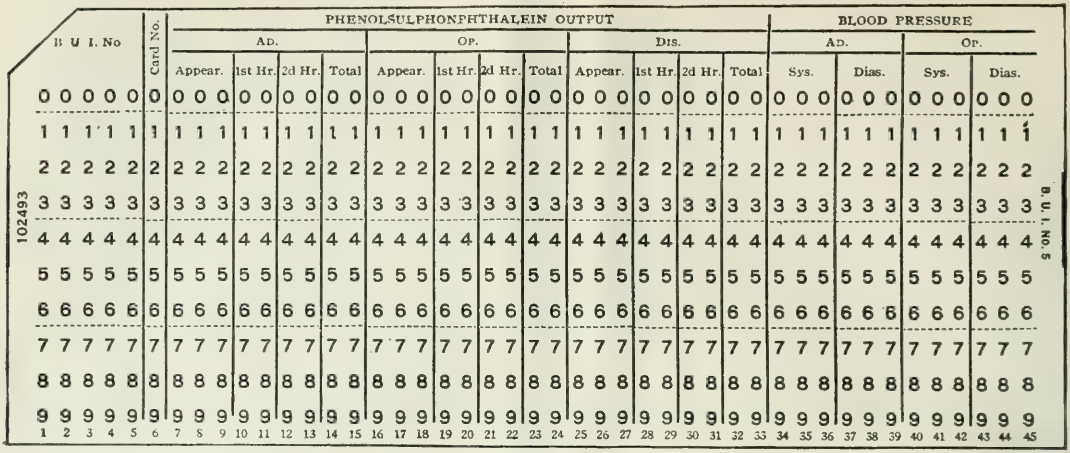


Fig. 9.

VI. An Example of Special Card Forms

As was pointed out earlier the recording of information on punched cards may be very advantageously employed for detailed records, extending in scope far beyond what needs to be included in routine hospital statistics. Suppose for example that one wishes to make a thorough analysis and study of 1000 consecutive operations of the same sort. Card forms can be devised to carry all of the most detailed minutiæ of the case histories, and then, when the cards have been punched, the information can be tabulated in every conceivable manner for study and discussion, with a minimum of labor. It is to be expected that as a properly organized statistical department in a hospital becomes well established more and more work of this sort will be demanded of it.

As an example of this sort of thing Figs. 6 to 10 inclusive are introduced to show in facsimile a series of card forms which are now in use in connection with a monographic study of 1000 consecutive prostatectomies, which have been performed by Dr. H. H. Young in the Brady Urological Institute of The Johns Hopkins Hospital. Without a detailed description of the cards, for which this is neither the time nor the place, it is sufficiently evident from the card headings that a literally enormous amount of detailed information is included and made readily available for statistical study of these cards.



B U I. No					Card No.	PHENOLSULPHONPHTHALEIN OUTPUT																											BLOOD PRESSURE											
						Ad.									Op.									Dis.									Ad.						Op.					
						Appear.			1st Hr.		2d Hr.		Total		Appear.			1st Hr.		2d Hr.		Total		Appear.			1st Hr.		2d Hr.		Total		Sys.			Dias.			Sys.			Dias.		
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
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9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45

102493

B. U. I. No. 5

Fig. 10.

Figs. 6–10 incl.—Facsimiles of card forms for recording in detail case histories of prostatectomy in the Brady Urological Institute.

VII. The Organization of the Statistical Department of a Hospital

It seems desirable to set out in some detail the plans of organization, in respect of both personnel and equipment, of what might be regarded as an ideal or model statistical department in a modern hospital, where special emphasis is laid upon the research and teaching aspects of the work. It is to be understood that the plan is meant to represent an ideal, and need not and probably at best would not be inaugurated *in toto,* but would gradually develop to something like the end here sketched.

A. *Personnel.*—The statistical staff should include:

(a) A chief statistician, who would direct the work of the department. This individual should have had a thorough training in the mathematical theory of statistics, in modern statistical organization methods, and have a well-digested background of the biological and medical sciences.

(b) An assistant statistician, with similar training, but presumably not yet with such a background of experience as the chief.

(c) A computer.

(d) A card punch operator for routine work. As occasion demands, extra key punch operators may be taken on as temporary appointments for the completion of specific large undertakings.

(e) A sorting and tabulating machine operator.

(f) A draftsman.

B. *Location and Space Requirements.*—The statistical department should be physically contiguous to the case history department of the hospital. The following space would appear to be a minimum requirement for proper functioning.

(a) Two offices, one for the chief and one for the assistant statistician.

(b) A large room for (1) the card files, (2) the sorting and tabulating machines, (3) the key punch operator.

(c) A large room for the computing and drafting room.

C. *Material Equipment.*—There would be required at least the following:

1. One card sorter. Considering the large use of routine cards for index purposes, two sorters would be a great advantage to avoid delay at times of peak load requirements. The routine key punch operator would operate the extra machine.
2. One tabulator (5 bank machine).
3. Three key punches.
4. Two listing adding machines.
5. Four calculating machines.
6. Miscellaneous calculating and drafting equipment.

An organization of the sort outlined could care for the routine statistical work of a large hospital, and at the same time make large contributions on the research side.

VIII. Conclusion

In this paper I have endeavored to show:

1. That modern statistical methods have a definite and distinct place in the functional economy of a hospital, and particularly of a hospital where research and teaching form a part of the activities of the institution.

2. That the inauguration of appropriate methods of handling hospital records will enormously facilitate and economize all the work of the hospital which directly or indirectly comes in contact with or depends upon case histories.

3. That to accomplish these ends only a relatively small addition to either personnel or material equipment is necessary.

AN ATYPICAL BACILLUS PARATYPHOSUS B INFECTION

By Hugh J. Morgan

(*From the Biological Division of the Medical Clinic of The Johns Hopkins Hospital and Medical School*)

Introduction

The purpose of this paper is to report the occurrence of what would seem to be an unusual infection in this country, and briefly to review the problems which arose in the attempt to classify bacteriologically the infecting agent. A clinical summary of the case will be given first, and this will be followed by a short discussion of the development of our knowledge of the group of organisms to which the causative agent belongs. An attempt will be made to classify it on the basis of our present knowledge of this group. The case to be reported is one of an atypical B. paratyphosus B infection.

Clinical History of Case

C. G., a colored male laborer, 45 years old, entered The Johns Hopkins Hospital, November 2, 1920, on the urological service, complaining of inability to void.

Family History.—Unessential.

Past History.—The patient remembers no illnesses except a rather severe attack of diarrhœa "several" years ago, which confined him to his bed for about a week. The stools were loose and contained mucus but no blood. He considers that his recovery was complete, although there has been a tendency to loose movements since, for which he thinks that sweets are responsible. He has never had typhoid fever, and on close questioning denies ever having had symptoms, other than the diarrhœa, which might be attributed to such an infection. He has received no typhoid vaccine.

He had gonorrhœa seven years ago. He has had nycturia (two or three times) for the past six or seven years, and this has gradually become worse during the development of the present illness.

Present Illness.—In 1913, following the attack of gonorrhœa mentioned in the past history, the patient developed a urethral stricture which became complete. It was necessary to use a catheter for some time. His condition gradually improved. Recently he noted that he was passing a continually smaller stream. Dysuria and hesitancy developed. Finally, during the month previous to admission, he became unable to void except with great effort, and then only a few drops at a time. Incontinence of urine resulted.

Physical Examination.—The patient was seen first in the genito-urinary dispensary where, after unsuccessful efforts had been made to pass the obstruction with catheters, he was advised to enter the hospital for operation.

On admission the general physical examination is reported as being essentially negative with the exception of a temperature of 99.5° F. which almost immediately became normal. An external urethrotomy was performed by Dr. William Jack and he was sent back to the ward from the operating room in good condition.

On November 3, the day following the operation, the temperature suddenly rose to 104° F. and there was marked tachypnœa. There was moderate abdominal distention. The spleen was not palpable. Blood examination: R. B. C. 3,784,000; W. B. C. 13,080; Hb. 70%. Differential W. B. C. Count: Pm. N., 65%; Pm. E., 1%; Small Mononuclears, 20%; large Mononuclears, 9%; Transitionals, 1%.

Urinary examinations.—S. G. 1.010. There was a heavy trace of albumin and numerous leucocytes. No casts or red blood cells. Guaiac reaction negative. Phthalein output 55% in two hours. *Blood urea:* 24 mgm. per 100 c. c. plasma. *Blood CO_2*: 40 Volumes per cent. *Blood cultures:* B. paratyphosus B (atypical); about 3 colonies per c. c. of blood. *Urine cultures:* B. paratyphosus B (atypical). *Stool cultures*—repeated on numerous occasions—negative for typhoid and paratyphoid organisms.

Course in Hospital.—The clinical picture presented by the patient was confusing to those seeing him daily. At times he was in a typical typhoidal state, at other times the picture was rather that of true sepsis. The leucocyte count ranged from 7880 to 17,000. The polymorphonuclear percentage varied from 69 to 83. The large mononuclear and transitional elements were fairly constantly around 10%; the small mononuclear were from 7% to 20%. The specific gravity of the urine varied between 1.008 and 1.015. Albumin was present (+). Many pus cells. Some mucus. No casts or red blood cells. The stools varied in number from 1 to 15 a day, soft—not especially characteristic microscopically. The guaiac test for blood was negative. The temperature for the first 11 days in the hospital was almost continuously high, usually ranging from 102°-104.5° F. On three occasions during that time it was as low as 99.5° F. After November 13 (eleventh day in the hospital) the curves became septic in type, the temperature almost reaching normal in the mornings and rising to 103° or 104° in the evenings. No bradycardia.

The following note is found in the history, dated December 2d (28th day of the disease): "Classical typhoidal state; dull; mental reactions very slow. Sordes on lips and teeth. Pulse soft. Respiration a little rapid and deep. Abdomen distended. Intestinal coils visible, chiefly in umbilical region. Liver not felt. Splenic dulness definitely increased.

"The continued bacteræmia, the soft stools, abdominal distention, the facies and mental aspect of the patient, the distinctly enlarged spleen, all suggest a general infection which, but for the irregular fever, would be characteristic clinically of typhoid. The history, high leucocyte count and high blood urea and urinary findings point to a pyelonephritis."

The tachypnœa which was so striking on admission disappeared a few days later. The patient ran a prolonged irregular febrile course, 57 days elapsing before a normal temperature was maintained for 24 hours. Blood cultures were positive during most of this interval. (Rough quantitative determinations were made with the following results: On the 2d day of the disease about three colonies were present in each cubic centimeter of blood cultured; 22d day, two colonies per c. c. of blood; 26th day, two colonies per c. c. of blood; 33d day, one colony per c. c. of blood; 42d day, one colony in 10 c. c. of blood; on the 50th day, and thereafter, the blood was sterile on culture.) There were no chills nor sweats. During the first three weeks of his stay in the hospital the patient was occasionally found in a drowsy state, but was easily aroused and would answer questions relevantly. After the third week of his illness he became mentally clear and was bright and cheerful.

On November 29th (25th day of the disease) B. coli was isolated by culture from the bladder urine. This was interpreted as evidence of a secondary urinary invasion by the latter organism, B. paratyphosus B having been previously isolated in pure culture. A marked secondary anæmia developed, the red blood cell count and hæmoglobin reaching as low as 2,300,000 and 42%. Considerable variation in the leucocyte count was present.

On the 35th day of the disease the following note was made: "Perineal fistula is practically closed—pin-point in size—but still exudes a little pus. The patient is much less drowsy than previously, and feels well." The spleen was not felt.

Forty-sixth day: "Still running a moderate fever. Urine contains numbers of pus cells. B. paratyphosus B continuously in blood. He has developed a secondary anæmia and seems to have lost some weight. Otherwise the general condition is good . . . . Spleen not

felt." On this same day blood urea and blood CO_2 determinations were made and found normal.

Forty-eighth day: Cystoscopic examination revealed an œdematous, congested bladder mucosa, without other abnormalities. On ureteral catheterization urine containing pus cells and B. coli was obtained from both sides. From the left kidney urine atypical B. paratyphosus B was also isolated. The diagnosis of the urologist making the examination was "bilateral pyelitis."

After the 50th day the temperature became lower in the evenings, rarely reaching 100.5°, and on the 58th day it remained normal. The urine at this time showed only an occasional pus cell and a faint trace of albumin. With the exception of three sharp rises in temperature, each lasting only a few hours, the chart was not remarkable thereafter.

With the disappearance of the fever and gross evidence of urinary infection the patient's general condition rapidly improved. On February 9th the urine showed only an occasional white blood cell and a small amount of mucus. His color and nutrition were rapidly approaching normal, and he was discharged feeling entirely well except for slight weakness.

Discussion

The case is of considerable theoretical interest from a diagnostic standpoint. Was it an example of B. paratyphosus B pyelitis and septicæmia—a well-known clinical entity recognized and described by Schottmüller,* or were we dealing with a paratyphoid pyelitis initiating a true paratyphoid fever by the production of a septicæmia? A third possibility (though it is highly improbable in this case) is that the pyelitis developed as a complication of paratyphoid fever.

The third possibility hardly warrants discussion. The conclusion that the illness was initiated by the development of an acute pyelitis seems unavoidable from the history. Moreover, there is nothing in the history, or the examinations made in the wards previous to operation, suggestive of preexisting paratyphoid fever. We come then to a consideration of the other two possibilities.

Was there a focal infection (pyelitis) with B. paratyphosus B which, by the continuous "breaking over" into the blood stream by the organism, finally brought about the more common generalized infection (paratyphoid fever)? We have not been able to find a proven case of such an occurrence in the literature. In his comprehensive review of the subject Schottmüller makes no mention of its occurrence. In our case the clinical evidence seems for the most part to be against this possibility. The classical typhoidal state was not constantly present. The spleen, though somewhat enlarged later in the disease, was never palpable and there were no rose spots. The temperature and pulse chart was essentially septic in type. Moreover, the absence of a leukopœnia with relative lymphocytosis is suggestive, though by no means conclusive, in the presence of a definite urinary infection. An intermittent diarrhœa was present but repeated examinations of the stools for B. paratyphosus B were negative. The prolonged septicæmia (the last positive blood culture was obtained on the 42d day of the disease) would seem to support this hypothesis to some extent—although the blood, as a rule, in paratyphoid fever becomes sterile after the third week.

* Schottmüller: Handb. d. inner. med., v. Mohr u. Staehelin.

We come then to the conclusion that the case is one of cystopyelitis of B. paratyphosus B origin, the most unusual feature of the condition being the prolonged septicæmia. The bacteræmia in itself is not unusual. It disappears as a rule, however, in from eight to twenty-one days. Moreover, in our case the rough quantitative determinations of the number of organisms in the blood stream at different stages of the disease are somewhat more suggestive of a general infection than of focal infection with incidental septicæmia. However, in the absence of other clinical and laboratory evidence of this condition (particularly the persistent absence of organisms in the stools) we do not feel that a diagnosis of paratyphoid fever is justifiable.

Historical Review of the Salmonella Group of Organisms

The etiological agent of the disease which has been reported above falls into the Salmonella group of bacteria, and it may not be amiss to review briefly the important features of this group with special reference to classification—in so far as it is possible to classify its members with the knowledge at hand.

By definition (Castellani) this genus is comprised of organisms which grow well on ordinary laboratory media; do not form endospores; are aërobes and often facultative anaërobes; without fluorescence, pigment formation or gelatin liquefaction; are without polar staining; are Gram-negative without a capsule; completely ferment glucose but do not ferment lactose; partially or completely ferment manitol, in addition to other carbohydrates. Milk is not coagulated.

The organisms falling under the above definition have been isolated for the most part during the past 25 years. The group has been extensively studied and its members classified on one basis or another, but as to their exact place within the group, and their relation one to another, relatively little is known.

In 1888 Gärtner[1] described a bacillus (B. enteritidis) isolated from beef which had caused a small epidemic of meat poisoning. The organism was found in the blood and spleen of a patient dying of the disease. Similar epidemics were described by Van Ermengem,[2] Holst,[3] Basenau[4] and others. De Nobele[5] in Belgium and Durham[6] in England described organisms giving rise to epidemics of gastroenteritis among groups of people in their respective countries. As a result of these more or less independent reports, a number of names for what is probably one and the same organism have been introduced into the literature. It is generally believed now that Gärtner's B. enteritidis, Basenau's B. bovis morbificans, B. morselle of van Ermengem, B. aertrycke of De Nobele and the organisms isolated and described by workers in similar epidemics are practically identical culturally and serologically—and some authorities feel that the name B. enteritidis (Gärtner) should be used exclusively for their designation.

In 1895 Gilbert[7] introduced the name "paracolon" to be applied to organisms occupying an intermediate place between the known typhoid and colon groups. In 1896 Achard and Bensaude[8] isolated from the urine of a patient with clini-

cally enteric fever an organism which they called a "paratyphoid" bacillus. Gwynn[9] in 1898 isolated a bacillus from an individual presenting the symptoms of typhoid fever, with cultural characteristics similar to those of B. enteritidis and which he called a "paracolon" bacillus. The patient's serum contained agglutinins for this organism but not for B. typhosus. A similar organism was isolated by Cushing[10] in 1900 from a costochondral abscess which had occurred during a "typhoid" convalescence. (The patient was said to have had a severe, though typical, attack of typhoid fever by her attending physician.) Cushing made an extensive study of the organism and concluded that it was an intermediate between B. typhosus and the colon group. In 1900 Schottmüller[11] obtained from five enteric patients organisms similar culturally to those of Achard and Bensaude, Gwynn and Cushing. By careful cultural and serological studies Schottmüller, Brion and Kayser showed that there were two distinct types present—one resembling the typhoid type (Paratyphosus A) the other resembling the enteritidis type (Paratyphosus B). Confirmatory findings were soon reported by other observers.

Other members of this group should be mentioned for the sake of completeness, and in order that a comprehensive view of the genus Salmonella may be obtained—B. suipestifer of T. Smith, B. typhi murium of Loeffler, B. icteroides of Sanarelli, B. psittacosis of Nocard and B. danysz.

Seiffert[12] in 1909, and Bainbridge[13] in 1910, considered the paratyphoid and allied organisms as a group. The latter concluded that the following classification could be made on cultural and serological grounds.

Salmonella group:
1. B. paratyphosus A.
2. B. paratyphosus B.
3. B. enteritidis (Gärtner).
4. B. suipestifer.

He (Bainbridge) felt that B. psittacosis, B. typhi murium, B. morbificans bovis, B. icteroides, B. danysz, and other organisms commonly placed in this group, are all identical with one or another of the four organisms listed above, or mixtures of them. He considered B. enteritidis (Gärtner) as identical with B. danysz, B. aertrycke and B. morbificans bovis; B. typhi murium as identical with B. suipestifer or B. enteritidis, or an admixture of B. enteritidis and B. suipestifer; B. psittacosis as identical with B. suipestifer.

Paratyphoid "C"

Bainbridge, in the discussion mentioned above, referred to a paratyphoid organism called "A" which, he stated, did not give the specific serum reactions with antiparatyphoid sera and which was antigenically different. It apparently did not fall into any of the groups discussed by him in his classification of the genus Salmonellæ. Zupnik[14] working with the organisms isolated by Cushing and Longcope in this country concluded that they belonged to a group different from either Paratyphosus A or Paratyphosus B. Messerschmitt[15] refers to an organism culturally a member of the paratyphoid group but which is different serologically from either B. paratyphosus A or B. In 1913 Uhlenhuth and Heubner[16] reported strains of organisms isolated from cases of hog cholera and from human feces which were similar to the paratyphoid organisms culturally, but which were inagglutinable in both anti-paratyphoid and anti-enteritidis sera. These organisms they called B. paratyphosus "C."

Since 1913 a number of writers have called attention to members of this paratyphoid-enteritidis group and a considerable literature has resulted. Particularly during the world war did these organisms come into prominence. In 1916 Hirschfeld,[17] chief bacteriologist for the Serbian Army, isolated an organism from the blood of a soldier who was thought to be suffering from paratyphoid fever. However, the organism was found to be serologically different from both B. paratyphosus A and B. Hirschfeld was able to recover, in all, 18 such organisms by blood culture from as many patients. He regarded it as a "new germ of paratyphoid fever" and called it B. paratyphosus "C." It was incorporated into the polyvalent antityphoid vaccine used by the Serbian Army. Working with a strain of Hirschfeld's B. paratyphosus "C," Dudgeon and Urquhart[18] prepared an antiserum which agglutinated the homologous organisms in dilutions of 1-20,000, but which did not agglutinate either B. typhosus, B. paratyphosus A or B. paratyphosus B. They absorbed this serum with all three of the latter named organisms (*i. e.,* B. typhosus, B. paratyphosus A, and B. paratyphosus B) and found that none of the homologous agglutinins were removed. However, they concluded that some paratyphoid "C" antigens have a definite paratyphoid B fraction. Castellani[19] in 1916 reported the recovery of six strains from enteric infections in the Adriatic-Balkan area which were similar culturally to the paratyphoid organisms but which were serologically distinct from them. Archibald, Hadfield, Logan and Campbell,[20] in 1916, reported similar findings at the Dardanelles. MacAdam[21] described a series of inagglutinable organisms isolated in Bagdad in 1918, morphologically and culturally indistinguishable from B. paratyphosus B, but serologically different. This worker concluded from serological studies that he was dealing with an organism distinguishable from both B. paratyphosus A and B, but closely related to B. paratyphosus B and B. aertrycke. Mackie and Bowen,[22] also working in Bagdad, report studies of several strains of organisms obtained by blood cultures from patients presenting, in the main, symptoms of the enteric group of diseases. From this work and observations subsequently made at the Lister Institute it is almost certain that they were dealing with organisms indistinguishable from Hirschfeld's B. paratyphosus "C." Kennedy[23] reports from India the isolation of five strains of an organism differing from both B. paratyphosus A and B, and probably identical with Hirschfeld's organism. Patton[24] records four strains of inagglutinable paratyphosus B in Egypt. No absorption tests are reported. Garrow[25] describes a strain from South Africa which was later found by Ledingham to be identical with Hirschfeld's. Broughton-Alcock[26] isolated by blood culture an organism differing from B. paratyphosus B in that it was non-motile and fermented dulcite only after repeated sub-

cultures. Serologically it reacted with B. paratyphosus B, and by absorption tests it was found to differ from B. aertrycke and B. gärtner. Dean[27] reports five strains from dysentery cases in Australia which apparently are quite similar to Hirschfeld's organism.

It is obvious that most of the reports cited above are from workers behind the Allied lines. It is interesting to note that German bacteriologists also met with atypical organisms which obviously belong to the group under discussion. Neukirch and Weil, in 1915, isolated an organism in Constantinople which they called B. ersindian. Culturally it resembled in all respects B. paratyphosus B, but proved to be definitely different serologically. It occurred in dysenteries. Weil later isolated it in Albania from patients without dysentery but presenting symptoms of sepsis. Dienes and Wagner reported similar cases from Eastern Europe. Levy and Schiff[28] saw 80 cases of this infection in Turkey and Palestine. In their article they recall Neukirch's classification of these infections into (1) septic, (2) typhoidal, and (3) dysenteric groups, and on account of the varying clinical pictures presented by the patients they raise the question as to whether or not the presence of the bacillus in the blood stream is not an accidental finding, just as B. suipestifer is in hog cholera.

In 1917 Weiss and Rice[29] reported the recovery of 15 strains of so-called B. paratyphosus C in this country and suggested that infection by it is more common than is generally recognized. In reviewing the literature we have met with no other reports of this group of organisms from workers in the United States.

Cultural Characteristics of the Paratyphoid Group

Culturally, B. paratyphosus A differs in but few respects from B. paratyphosus B. The former usually grows scantily on potato while with the latter the growth is abundant. B. paratyphosus B in litmus milk produces a primary acidity which rapidly changes to an alkaline reaction, as contrasted with the prolonged acidity of B. paratyphosus A. Krumweide, Pratt and Kohn[30] found that, while for the most part this statement holds, a moderate number of A and B types give intermediate reactions, so that the two groups merge. Jordon[31] concludes that this difference between the para A and B types in rapidity of alkalinization of milk is largely, if not altogether, a numerical relation due to gradations in the amount and rate of multiplication of the organisms. Burnet and Weissenbach[32] note that B. paratyphosus B reduces lead acetate (H_2S production), causing a darkening of the agar to which it has been added, thus differentiating it from B. paratyphosus A. Krumweide, Pratt and Kohn[33] found that 50 paratyphosus A strains and 14 paratyphosus B strains were sharply differentiated by the reduction and non-reduction of fuchsin from glucose-serum-water media. (Paratyphosus A caused reduction and Paratyphosus B caused no reduction.) These same observers[34] found that with their strains, which were agglutinatively A, xylose fermentation did not occur.

From the above review of the cultural differences of two fairly fixed types as B. paratyphosus A and B, it would be surprising indeed to find that organisms, so closely allied to B. paratyphosus B as members of the so-called C group, possessed distinctive cultural characteristics; and as a matter of fact they do not. No mention is made in the literature of the existence of any cultural differences between B. paratyphosus B and this recently described group, with one exception. Weiss and Rice[29] noted that none of their 15 strains produced gas on inosite, whereas, of 25 strains of B. paratyphosus B, 19 produced gas within 18 hours, while 6 produced no gas at the end of 6 days. The organism recovered from our case does not ferment inosite.

Serological Characteristics of the Group

It is evident from the discussion of the cultural characters of the paratyphoid group that differentiation of its members on that basis is uncertain. Serological methods are employed, and it was by the use of these methods (specific antisera and Castellani's agglutinin-absorption test) that the investigators cited above were led to believe that in the so-called B. paratyphosus C they were dealing with a third form of paratyphoid organism. However, their results varied considerably. Some strains were altogether inagglutinable by specific agglutinating serum for B. typhosus, B. paratyphosus A, B. paratyphosus B and B. enteritidis, but agglutinated in high dilutions of serum prepared by the use of so-called paratyphosus C antigens. Other strains reported were agglutinated by one or more of these specific antisera, but only in high concentrations. By the use of the agglutinin-absorption test in practically every instance it was shown that this agglutination was due to the presence in the sera used of co-agglutinins, and that absorption of the latter with the bacillus under investigation (B. paratyphosus C) did not alter the serum's agglutination-titer for its homologous organism.

The fact that the organisms reported show considerable variations to specific sera, together with the fact that on animal inoculation many strains produce, in considerable concentration, co-agglutinins for allied groups of organisms, while others do not, suggests immediately the possibility that we are not dealing with a single organism representing a new type but with a group of organisms, closely related to one another. Since all of the strains reported are allied to B. paratyphosus B, it is not improbable that the so-called paratyphosus C group represents variants of B. paratyphosus B, some being almost identical with the mother-type, while at the other extreme B. enteritidis is approached. Schutze[35] working at the Lister Institute made a critical study of the entire paratyphoid group with agglutinin-absorption tests. In this work he dealt with many of the strains referred to above. He concluded, on the basis of his findings, that the Hirschfeld strain is a true member of the paratyphoid B group, and that the term "paratyphoid C" should not be used in connection with it and similar organisms. He insists that the latter term was introduced into the literature by Uhlenhuth

to designate an organism which had "no agglutinogenic relationship, active or passive, to B. paratyphosus B and that, while some members of the Hirschfeld series may be entirely inagglutinable by para B sera, "others do respond to a greater or less extent, and all give sera which agglutinate B. paratyphosus B to a considerable degree." (Although Schutze did not work with any of the strains isolated by German workers, the description of the organisms isolated by the latter, as well as the proximity of the places of isolation, make it quite clear that the British and German workers were dealing with similar or closely allied infections. It would therefore seem highly probable that these strains would also fall into Schutze's large paratyphosus B group.)

Description of the Organism

The organism isolated in the case reported in this paper was first recovered in blood culture November 3, 1920. It proved to be a Gram-negative, mobile bacillus and a prolific grower on the ordinary laboratory media. Litmus milk was first acidified (24 hrs.) and then immediately alkalinized (48 hrs.). Its action on the various sugars is as follows:

Glucose Levulose Mannite Galactose	Acid and gas.	Saccharose Lactose Inulin Raffinose Inosite Dulcite	No acid. No gas.

There is no production of indol. Media containing lead acetate are darkened within 24 hours. Gentian violet[38] (1-10,000) does not inhibit its growth. Glucose-serum-water is turned light pink color and a scant, fine precipitate occurs.

Culturally, then, we found that we were dealing with an organism of the paratyphoid group, and probably of the B. paratyphosus B division.

Serologically its identity was uncertain. With the first antiparatyphoid sera used we recorded the following results:

"H L" Antiparatyphosus A serum: No agglutination.

"H L" Antiparatyphosus B serum: Agglutination 1-150.

At this time the patient's serum agglutinated a killed culture of B. paratyphosus B in dilutions of 1:50. There was no agglutination with B. paratyphosus A.

TABLE I

	Before absorption	After absorption with Bacillus "X"
"A. T." Serum * v. B. paratyphosus B (N. Y.)	Agglutination 1–2,500	Agglutination 1–2,500
	Before absorption	After absorption with B. paratyphosus B (N. Y.)
"A. T." Serum v. Bacillus "X"	Agglutination 1–1,000	No agglutination

* A polyvalent antityphoid serum containing agglutinins for B. typhosus, B. paratyphosus A and B. paratyphosus B.

The low titer of our antiparatyphosus B serum against the unknown, together with the knowledge of the fact that organisms apparently falling into the paratyphosus B group are notoriously deceptive as to their identity, indicated the necessity of employing other methods for its identification. Castellani's absorption method was used. For purposes of simplification the organism under discussion will be called Bacillus "X" throughout the remainder of this report.

Thus it was shown that Bacillus "X," while agglutinated by the specific paratyphosus B fraction of the "A. T." serum used, was unable to remove the paratyphosus B agglutinins from the serum.

To confirm the above findings similar tests were made with two different monovalent antiparatyphosus B sera, with the following results:

TABLE II.—ANTIPARATYPHOSUS B SERUM NO. 684

	Before absorption	After absorption with Bacillus "X"
Serum No. 684 v. B. paratyphosus B (N. Y.)	Agglutination 1–1,280	Agglutination 1–1,280
	Before absorption	After absorption with Bacillus "X"
Serum No. 684 v. Bacillus "X"	Agglutination 1–1,280	No agglutination

TABLE III.—ANTIPARATYPHOSUS B SERUM NO. 999

	Before absorption	After absorption with Bacillus "X"
Serum No. 999 v. B. paratyphosus B (N. Y.)	Agglutination 1–2,560	Agglutination 1–2,560
	Before absorption	After absorption with B. paratyphosus B (N. Y.)
Serum No. 999 v. Bacillus "X"	Agglutination 1–1,280	No agglutination

(In each of the experiments detailed above, and in those which are to follow, a salt and serum control was run to reveal "spontaneous agglutination" if present. Absorption was proved to be complete in each case before the cross-agglutination tests were run.)

From the above protocols it is evident that the same phenomenon met with in the use of the "A. T." serum is also present when the monovalent paratyphosus B sera are used, that is to say, Bacillus "X" is unable to absorb out the specific paratyphosus B agglutinins from the sera, while B. paratyphosus B constantly absorbs out the co-agglutinins present in the sera for Bacillius "X." Nevertheless, in considering the results of these experiments, it is noteworthy that Bacillus "X" is agglutinated almost to the full titer of the serum for B. paratyphosus B.

To investigate further the validity of the assumption of the presence of co-agglutinins in the above sera as the explanation of the agglutination of Bacillus "X" by them, and also to

study the antigenic properties of the organism the following absorption experiments were made with the patient's serum:

TABLE IV

Patient's serum *v.* B. paratyphosus B (N. Y.)	Before absorption	After absorption with Bacillus "X" (homologous organism)
	Agglutination 1–160	No agglutination
Patient's serum *v.* Bacillus "X" (homologous organism)	Before absorption	After absorption with B. paratyphosus B (N. Y.)
	Agglutination 1–320	Agglutination 1–80

The above experiment was repeated, another strain of B. paratyphosus B (Sch. of H.) being employed, with similar results. Careful controls were run to guard against misinterpretation through "spontaneous agglutination" and incomplete absorption.

From the above experiment it would seem that we are dealing with two organisms antigenically different. (In spite of the low titer of the patient's serum we believe it permissible to utilize his serum as one showing specific immunity [agglutinins] for the organism being studied.) Here, too, the phenomenon of group agglutination is quite apparent.

Similar experiments were performed with the patient's serum and B. enteritidis (Gärtner) and B. suipestifer. The latter organism showed no agglutination whatever. The protocol of the former experiment is given:

TABLE V

Patient's serum *v.* B. enteritidis	Before absorption	After absorption with Bacillus "X" (homologous organism)
	Agglutination 1–160 (*atypical)	No agglutination
Patient's serum *v.* Bacillus "X"	Before absorption	After absorption with B. enteritidis
	Agglutination 1–320	Agglutination 1–320

* These tubes showed a finely granular appearance instead of a flocculent precipitate as in the case of Bacillus "X."

Substituting two known antiparatyphosus B sera for the patient's serum the above experiment was repeated with B. enteritidis. (B. suipestifer was not agglutinated by either of the antiparatyphosus B sera used.) The results in each case indicated clearly that the agglutinins present in these sera for B. enteritidis were group agglutinins, and that their removal had no appreciable effect on the agglutination titer of the sera for B. paratyphosus B. Moreover, they were removed when the sera were absorbed by B. paratyphosus B.

In order to study further the antigenic and serological characteristics of this organism a normal rabbit was immunized by the intravenous introduction of graduated doses of a killed broth culture of Bacillus "X," resuspended in salt solution. The immunization resulted in a serum which agglutinated the homologous organism in dilutions of 1-2400. At the same time a second rabbit was immunized to our stock strain of B. paratyphosus B (N. Y.). The second serum has a titer for the homologous organism of macroscopic agglutination in dilutions of 1-2400.

As in the above experiments agglutination, cross-agglutination and absorption tests were performed. The tabulated results follow:

TABLE VI

	Bacillus "X" immune serum	B. paratyphosus B immune serum
B. paratyphosus B	Aggt. 1–640	Agglutination 1–2,560
Bacillus "X"	Aggt. 1–2,560 ..	Agglutination 1–160
B. suipestifer	Aggt. 1–320	
B. enteriditis	Aggt. 1–160	

The Bacillus "X" produced in the rabbit's serum, as in that of the patient infected, agglutinins for closely allied organisms. The absorption-tests which follow (Tables VII, VIII and IX) indicate that, as in the other serum, these are co-agglutinins the removal of which, by absorption of the serum with these organisms, has no effect on the titer for the homologous strain.

TABLE VII

Bacillus "X" antiserum *v.* Bacillus "X"	Before absorption	After absorption with B. paratyphosus B (N. Y.)
	Agglutination 1–2,560	Agglutination 1–1,600+
Bacillus "X" antiserum *v.* B. paratyphosus B (N. Y.)	Before absorption	After absorption with Bacillus "X"
	Agglutination 1–640	No agglutination

TABLE VIII

Bacillus "X" antiserum *v.* Bacillus "X"	Before absorption	After absorption with B. suipestifer
	Agglutination 1–2,560	Agglutination 1–1,600+
Bacillus "X" antiserum *v.* B. suipestifer	Before absorption	After absorption with Bacillus "X"
	Agglutination 1–320	No agglutination

TABLE IX

Bacillus "X" antiserum *v.* Bacillus "X"	Before absorption	After absorption with B. enteritidis
	Agglutination 1–2,560	Agglutination 1–1,600+
Bacillus "X" antiserum *v.* B. enteriditis	Before absorption	After absorption with Bacillus "X"
	Agglutination 1–160	No agglutination

A similar absorption experiment was performed with the antiparatyphosus B (N. Y.) serum.

TABLE X

Antiparatyphosus B (N. Y.) serum *v.* B. paratyphosus B (N. Y.)	Before absorption	After absorption with Bacillus "X"
	Agglutination 1–2,560	Agglutination 1–2,560
Antiparatyphosus B (N. Y.) serum *v.* Bacillus "X"	Before absorption	After absorption with paratyphosus B (N. Y.)
	Agglutination 1–160	No agglutination

Here also it is obvious that the organisms are antigenically different.

SUMMARY

(1) An organism was isolated from the blood of a patient who initiated his general infection by the development of a cystopyelitis (the latter following urethral stricture). A bacteræmia persisted over six weeks. A septic fever was present with leucocytosis. The organism was recovered on numerous occasions from the urine and blood, but never from the stool. With the cure of the urethral stricture and the subsidence of the cystitis and pyelitis the bacteræmia disappeared and the patient apparently made a complete recovery. The condition is an example of a clinical entity infrequently encountered but fully described by Schottmüller.

(2) The organism morphologically and culturally corresponds to B. paratyphosus B, yet serologically and antigenically it shows marked differences from the stock strains of B. paratyphosus B.

(3) It belongs to a large group of organisms which has recently come into prominence as a cause of disease (particularly among soldiers), and whose geographical distribution has been limited for the most part to the eastern hemisphere. Members of this group have been called by those isolating them "B. paratyphosus C," "Inagglutinable B. paratyphosus B," "Paracolon Bacillus," etc., since they all show, to a more or less degree, definite serological differences from B. paratyphosus B. Some have been shown to differ antigenically from the latter organism.

(4) A number of these strains (all of those studied by Schutze[35]) give sera which agglutinate B. paratyphosus B to some extent. When considering the members of the group individually, one finds strains showing only slight differences, those showing moderate differences and those showing extreme differences from the accepted B. paratyphosus B.

CONCLUSIONS

(1) The organism described in this communication is a member of the group which has rather loosely been called "paratyphosus C."

(2) Careful study has shown that this organism is closely related to B. paratyphosus B.

(3) It would not seem justifiable to employ the term "Paratyphosus C" in connection with this organism; it should rather be considered a variant of the B. paratyphosus B originally described by Schottmüller.

I wish to express my appreciation for the many valuable suggestions given me by Dr. A. R. Dochez, Chief of the Biological Division of the Medical Clinic of The Johns Hopkins Hospital and Medical School.

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GROWTH REQUIREMENTS OF INFLUENZA BACILLI

By T. M. Rivers and A. K. Poole

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Pfeiffer[1] discovered a new group of bacilli; he said that they were hemoglobinophilic and the cause of influenza. Since then much has been done to prove or disprove the various claims made for these bacilli by their discoverer. The controversy over the etiology of influenza will not be discussed here. Some, however, of the most significant work upon the hemoglobinophilic qualities of these bacilli will be cited and further evidence obtained in this laboratory bearing upon the subject will be submitted in this paper.

Grassberger[2] could not obtain a growth of influenza bacilli on media that contained hematin instead of hemoglobin except in symbiosis with other bacteria. Cantani[3] claimed he could grow them on media enriched with spermatic fluid which did not give the spectroscopic bands of hemoglobin. Ghon and Preyss[4] considered hemoglobin necessary even though it were present in such small quantities that it failed to give the bands with the spectroscope unless hydrazin were added. Nevertheless, they grew influenza bacilli on media containing hematin in symbiosis with other bacteria. Neisser[5] was able to grow influenza bacilli, isolated from a case of purulent conjunctivitis, on nutrient agar for 20 generations in symbiosis with a xerosis bacillus. Rivers[6] reported that he could grow both the hemolytic and non-hemolytic forms of these bacilli for a number of generations on hemoglobin-free media in symbiosis with other bacteria. Putnam and Gay[7] were unable to confirm Neisser's work. Davis[8] thought that hemoglobin acting as a catalytic agent was necessary for growth, and showed that a very small amount was required (1 part in 180,000 parts of medium). According to him, growth will take place in the presence of coagulated hemoglobin, but if the hemoglobin be broken up by excessive heating into hematin and globin no growth occurs. Fleming[9] was the first to offer proof that seriously interfered with the generally accepted idea, that hemoglobin is essential for the growth of influenza bacilli, by securing a good growth upon agar to which had been added a small quantity of fluid obtained by digesting blood with an equal amount of normal sulphuric acid and then neutralizing this with normal caustic soda. Olsen[10] obtained a growth of influenza bacilli on media containing hematin or hemin in symbiosis with other bacteria. Fildes[11] states that hemoglobin, unless changed, actually inhibits the growth of influenza bacilli and that there are two substances essential for the growth of these organisms. He is probably correct in saying that there are two factors, but his explanation based on the oxygen requirements of the bacilli is not necessarily true. Thjotta and Avery[12] consider two substances as essential for the growth of these bacilli. Both are in blood, one is resistent to excessive heating, the other is not. They do not think hemoglobin as hemoglobin to be one of the factors and also find the heat-labile substance in a number of bacteria and vegetables.

For several years to one of the authors (Rivers) the phenomenon of augmented growth of influenza bacilli in the vicinity of certain other bacteria under various conditions, called symbiosis, has been very interesting and seemed to hold in some way the secret of the real growth requirements of these bacilli. A study of this phenomenon of symbiosis has answered many questions and has shown that influenza bacilli are probably not hemoglobinophilic.

Augmented growth of influenza bacilli in the vicinity of other bacteria is more marked on human or hen blood agar plates than on rabbit, cat, dog or pigeon blood agar plates. In 1919 Rivers[13] showed that on 5 per cent fresh human blood agar a poor growth of influenza bacilli occurred, whereas on 5 per cent fresh rabbit or cat blood agar an abundant growth was obtained. An inhibitory substance, which did not exist in fresh rabbit serum, was found in fresh human serum, was removed by heating the serum for half an hour at 56° C., and was restored by adding a small quantity of fresh rabbit serum which in itself was not inhibitory. The phenomenon of symbiosis was marked on 5 per cent fresh human blood agar, whereas this was not true when rabbit or cat blood was used. The addition of the human blood to hot agar (95° C.) destroyed the inhibitory substance, allowing a splendid growth, and the picture of symbiosis sank into the background. Since then hen blood has been found to act similarly to human blood, while dog and pigeon bloods are like cat and rabbit bloods. The explanation for this phenomenon on fresh human blood agar and not on fresh rabbit blood agar was that the symbiotic bacteria removed in some way the inhibitory substance, just as heat does, allowing the influenza bacilli to grow best in their vicinity. This is only a partial explanation for the phenomenon as it occurs on blood agar and does not explain at all the fact that influenza bacilli can be grown on meat-infusion agar in symbiosis with other bacteria.

The change in hydrogen-ion concentration of the medium in the vicinity of the symbiotic bacteria next suggested itself as one of the factors entering into the picture observed on blood agar or meat-infusion agar plates. By some it is contended that this explains fully the augmented growth of influenza bacilli when grown with other organisms. To meat-infusion agar, pH 6.8, 7.6 and 8.6, was added 2.5 per cent fresh rabbit blood and 1 per cent glucose. Plates were made and inoculated with influenza bacilli. In the center of the plates, the three hydrogen-ion concentrations being used for each organism, were streaked *B. coli*, *B. alkaligenes*, and *Staphylococcus aureus*. The staphylococci and colon bacilli fermented the glucose and inhibited completely the growth of influenza bacilli near them on the plates with pH 6.8 and 7.6 (hydrogen-ion determinations were made before the blood was added). On the most alkaline plate, pH 8.6, the growth was best near the streak of colon bacilli, probably because of a

change in the hydrogen-ion concentration to a more favorable one for the growth of influenza bacilli. The opposite occurred on the plates streaked with *B. alkaligenes*. The growth of influenza bacilli was best near the streak of *B. alkaligenes* on the plates with pH 6.8 and a complete inhibition of growth occurred near the streak on the plate with pH 8.6. The blood near the center streaks was hemolysed and it could be seen that the hydrogen-ion concentration had been changed when the plates were flooded with brom-cresol purple. The striking thing, however, was that often just outside the zone of inhibition another zone of remarkably augmented growth occurred, reading thus: Streak of staphylococci near which would be no growth of influenza bacilli, then an unusually good growth, and then the usual growth on the plate at that hydrogen-ion concentration. Some of the phenomenal growth just beyond the zone of inhibition probably can be explained on the supposition that the hydrogen-ion concentration at that point and the action of the acid or alkali on the blood produce something more suitable for the use of influenza bacilli or liberate something in the process of breaking up the blood cells. This will be spoken of later.

Alkalies and acids were used for the center streaks to see if they would give the same results as bacteria. Blood agar plates of different hydrogen-ion concentrations were made as above and inoculated with influenza bacilli. Then acid or alkaline agar at 45° C. was placed on the center of the plates. Near these streaks the blood was hemolyzed or made brown and the same picture of inhibition and augmentation in the growth of the influenza bacilli was obtained as when bacteria were used, except that outside the zones of inhibition the areas of augmented growth, although present, were not so marked.

The fact that the zones of augmentation were more marked when bacteria were used suggested that they did not occur solely from an alteration in the blood, from the liberation of some substance in the blood cells, or from a change in the hydrogen-ion concentration, but in some way were influenced by the symbiotic bacteria. This is further confirmed by the fact that in growing influenza bacilli on meat-infusion agar in symbiosis with other bacteria the growth always occurred in the influenzal transplant nearest the symbiotic bacterial transplant. Sometimes the two transplants were half an inch to an inch apart. Whatever this substance may be, it is diffusible and reached the influenza bacilli by diffusion through the medium rather than by being carried to them by way of the air. This diffusible substance was considered one of the factors producing augmentation of growth of influenza bacilli near other bacteria and possibly a necessary factor for the growth of these bacilli under all conditions.

As it seemed possible that, in different bacteria or liberated by them in the process of growth, there is something which will augment the growth of influenza bacilli under various conditions, we added the extracts of bacteria to the media instead of making symbiotic cultures. Yeast was used first because it could be obtained easily in large quantities. One cake of bakers' yeast was mixed with 50 c. c. of distilled water, boiled one minute, filtered, and sterilized through a Mandler filter. To 100 c. c. of meat-infusion broth 15-20 c. c. of this yeast extract was added. This mixture was tubed in 10 c. c. quantities and was incubated to be sure of its sterility. When this medium was inoculated with influenza bacilli a good growth was obtained. The cultures were carried for 10 generations and discontinued. The first tube was inoculated with a loop of bacteria from a blood agar slant. Subsequent transplants were made by using 0.5 c. c. of the broth cultures. The bacilli in the tenth generation were growing normally, looked as usual under the microscope, formed indole, and were in pure culture when plated. When autoclaved yeast extract was used instead of that sterilized through a Mandler filter the medium was found unsuitable for the growth of influenza bacilli. The meat-infusion broth was made in the usual way and had been autoclaved 15 minutes under 15 pounds of pressure. These facts indicate that in yeast there is something essential for the growth of influenza bacilli and that it can be destroyed in the autoclave under 15 pounds of pressure for 15 minutes.

At this point the problem seemed an easy one, but soon it was observed that only certain lots of meat-infusion broth supported growth of influenza bacilli for many generations when the unautoclaved yeast extract was added. Another substance also appeared necessary for their growth and sometimes it was present in meat-infusion broth, at other times absent. Interest was fixed upon this second factor in attempts to find out what it was.

In looking over some experiments, several interesting facts were observed. Agar made with 2 per cent peptone and 0.5 per cent sodium chloride permitted growth of influenza bacilli only when more than 2.5 per cent fresh rabbit blood was added while the agar was at 45° C., and yet allowed a good growth when 0.5 per cent blood was added while the agar was 95° C. One drop of blood added to 100 c. c. of meat-infusion agar at 45° C. permitted little or no growth of the bacilli. If the agar were at 95° C. when the drop of blood was added a fairly good growth occurred. This was striking. So little blood, one drop in 100 c. c. of medium, certainly was not inhibitory because of some deleterious substance, inasmuch as 5 per cent of the same fresh blood with the same kind of agar allowed an abundant growth. Apparently in 5 per cent fresh unheated rabbit blood there was free enough growth-producing substances; while in one drop of unheated blood in 100 c. c. of agar not enough was supplied. Heating this one drop of blood to 95° C made available sufficient growth-producing substances. Others find that this alteration in the blood can be made with acids or pepsin as well as by heat.

The autoclave stable factor which at times was present in meat-infusion broth was sought for in blood. A clot from 500 c. c. of blood was infused with 200 c. c. of physiological saline solution, boiled, filtered, and autoclaved half an hour under 15 pounds of pressure. This autoclaved extract of blood clot was slightly brownish but did not give the spectroscopic bands of hemoglobin. An extract of yeast was prepared, half of which was sterilized through a Mandler filter, the other half in the autoclave. Two per cent peptone water

or agar was used as the basic medium. Influenza bacilli did not grow on these alone. If enough unheated rabbit blood, or "chocolate blood" were added, a good growth occurred. If 15-20 c. c. of the autoclaved blood clot extract, the filter-sterilized yeast extract, or the autoclaved yeast extract, were added alone to the peptone water or agar, no growth was obtained. If the autoclaved blood clot extract and the autoclaved yeast extract were added, there was no growth. If, however, the filter-sterilized yeast extract was used with the autoclaved blood clot extract, an abundant growth was secured for as many generations as desired.

When these facts are analysed, two substances at least are shown to be essential for the growth of influenza bacilli. Both of these are in blood; one resists autoclaving for 30 minutes under 15 pounds pressure, the other does not. The autoclave labile factor is found also in yeast. The autoclave stable one is not hemoglobin, if the spectroscopic examination or if autoclaving the blood clot extract half an hour be proof enough. So far this last factor has not been found outside of blood and may come from blood pigment even though it is not hemoglobin itself. In making media it has been noticed that some meats are bloodier than others. Certain lots of meat-infusion broth must contain enough of the autoclave stable substance from blood, as the growth of influenza bacilli is supported when unautoclaved sterile yeast extract is added. This explains the phenomenon of symbiosis on meat-infusion agar. The autoclave stable substance from the blood is in the agar and the autoclave labile factor is furnished by the symbiotic bacteria.

CONCLUSIONS

1. The phenomenon of augmented growth of influenza bacilli in the vicinity of other bacteria on solid media may be due to any one or, at times, all of the following factors:

(a) The removal of inhibitory substances that are marked in certain bloods, as human and hen's blood.

(b) The change of the hydrogen-ion concentration to one more favorable for the growth of influenza bacilli.

(c) The alteration in the blood, making growth substances more available.

(d) The production by the symbiotic bacteria of an autoclave labile substance necessary for the growth of influenza bacilli.

2. Two substances are essential for the growth of influenza bacilli. Both are in blood. One resists autoclaving half an hour under 15 pounds pressure, the other does not. The autoclave stable substance is not hemoglobin, although it may be derived from the blood pigment, and as yet has not been found outside of blood. The autoclave labile substance has been obtained also from yeast.

3. In what way these two factors operate to promote the growth of influenza bacilli is not known.

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PUBLICATIONS

THE GRADUAL WITHDRAWAL METHOD OF TREATING MORPHINISM: A MATHEMATICAL NOTE[1]

By JOHN RICE MINER

In the treatment of morphinism by gradual decrease of the dosage of morphine administered, a common procedure is to fill a bottle with distilled water to which is added a certain quantity of morphine. Enough of this mixture is administered as a first dose so that the morphine content will approximate the amount to which the patient has been accustomed. After removal of the dose it is replaced by an equal volume of distilled water. The second and subsequent doses are of

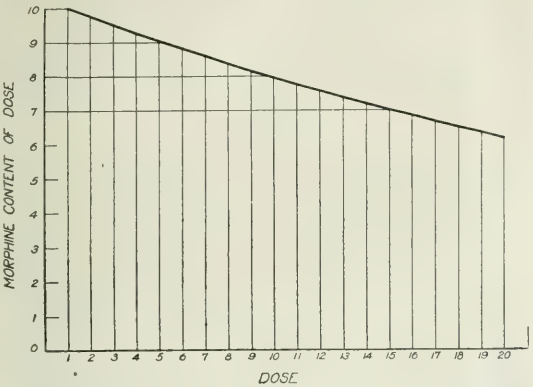

FIG. 1.—Morphine content of successive doses. The curve appears at first glance to be a straight line but it is really slightly convex to the base.

the same volume as the first, but as each time enough water is added to maintain the original volume of the mixture, the morphine content of the doses diminishes steadily. The question has been raised by one of the clinical departments of the hospital, as to the amount of morphine contained in the n^{th} dose, where n may have any value, as for example, 4, 5, or 10.

Let there be originally M milligrams of morphine in solution in L c. c. of water, and let the one dose of the *solution* be $\frac{1}{k} \times L$ c. c. Let U_n represent the total amount of morphine present in the L c. c. of solution after $n-1$ doses have been taken out (*i. e.,* just before the n^{th} dose), and D_n the amount of morphine in the n^{th} dose.

$$D_i = \frac{U_i}{k}$$

since each dose is $\frac{1}{k}$ of the whole solution.

But

$$U_{i+1} = U_i - D_i = U_i \left(1 - \frac{1}{k}\right).$$

Therefore by mathematical induction, since

$$U_1 = M$$

$$U_2 = M\left(1 - \frac{1}{k}\right)$$

$$U_3 = M\left(1 - \frac{1}{k}\right)^2$$

....

$$U_n = M\left(1 - \frac{1}{k}\right)^{n-1}$$

For example, suppose $M = 400$ mg. of morphine, $L = 40$ c. c. and $\frac{1}{k} = \frac{1}{40}$, so that each dose is 1 c. c. in volume. Fig. 1 shows the morphine content of each dose from the first to the twentieth under these conditions.

At the tenth dose the morphine content is diminished by only one-fifth, and even at the twentieth dose by less than two-fifths. The decrease in dosage is much slower than might be supposed on first thought. A stage where the dosage of drug would be $\frac{1}{10}$ of the original dose would occur only at the 92d dose in the series. Incidentally it should be noted that the morphine would never entirely disappear from the bottle.

[1] Papers from the Statistical Department of The Johns Hopkins Hospital, No. 2.

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The Hospital Bulletin contains details of hospital and dispensary practice, abstracts of papers read and other proceedings of the Medical Society of the Hospital, reports of lectures, and other matters of general interest in connection with the work of the Hospital. It is issued monthly. Volume XXXII is in progress. The subscription price is $4.00 per year.

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THE FORMATION OF VACUOLES AND NEUTRAL RED GRANULES IN CONNECTIVE-TISSUE CELLS AND BLOOD CELLS OBSERVED UNDER ABNORMAL CONDITIONS

By Rosa E. Prigosen

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Introduction

The accumulation in the cell of granules and vacuoles having a special affinity for neutral red is a phenomenon that has been studied in the living animal by Ehrlich (1894) in amphibia, by Metchnikoff (1907) in protozoa, by Fischel (1901) in the salamander, by Bethe (1909) in the rhizostoma, by Policard (1910) in the trypanosomes, by Coghill (1915) in the amphibian embryos, by Shipley (1916) in the trypanosomes, in the eggs of cerebratulus and in chick embryos by M. R. Lewis (1917, 1920), and by Clark and Clark (1918) in the tadpole. Numerous observers have also described these bodies in tissue cultures. Sundwall (1917), Matsumoto (1918) and Shipley (1919) used plasma cultures; Lewis and Lewis (1915), W. H. Lewis (1919), Hogue (1919), M. R. Lewis (1920), Smith (1920), and Matsumoto (1920) studied cultures in Locke-Lewis solution. Other investigators, describing the behavior of connective-tissue films (Renaut, 1907) and blood cells (Plato, 1900; Cowdry, 1914) have discussed certain neutral red bodies found in the cells. Cowdry, Gatenby, Duesberg, and others, in investigating the various structures of the cytoplasm, have incidentally referred to these granules. This work, initiated by Ehrlich and extended by numerous subsequent observers, has given rise to various theories and interpretations concerning the origin and functional activity of these granules and vacuoles.

At the suggestion of Prof. and Mrs. Lewis, a study of cells under abnormal conditions was undertaken in order to determine, if possible, whether altering the environment of the living cell in such a manner as ultimately to cause its death would affect the behavior of the cell toward neutral red and janus green.

Method and Material

Film preparations of subcutaneous connective tissue of 8- to 17-day chick embryos were made. The tissue covering the inguinal glands and legs yields the thinnest layer of fibroblasts and in the older chicks is of such a consistency that it can be easily manipulated on the slide. The piece of tissue, about 1 mm. square, excised from this region was carefully stretched upon a slide in a drop of plain Locke's solution, or in Locke's solution containing neutral red (1-10,000), and aerated for a few seconds before being flattened with a cover-slip. Preparations in these two media were made at the same time. Some of each were sealed to exclude all oxygen. The preparations in Locke's solution were later stained with neutral red.

A film of connective tissue affords an opportunity for studying several kinds of cells—fibroblasts, clasmatocytes, and erythrocytes. The boundaries of the fibroblasts are not readily seen, but when defined are more or less spindle-shaped. These cells contain a few fat bodies and also mitochondria arranged somewhat radially at one side of the nucleus. The fat globules are highly refractive and can thus be distinguished from the subsequent degeneration products. The mitochondria, usually rod-shaped, occasionally assume a spherical or vesicular form, simulating somewhat the degeneration granules and vacuoles; from these structures, however, they can be differentiated by the use of the janus green or janus black.

Experiments

The experiments were grouped into seven series, each of which, with the exception of No. 6, was repeated many times. (1) Unsealed preparations of embryonic chick tissue in Locke's solution containing about 1-10,000 neutral red. (2) Sealed preparations of embryonic chick tissue in Locke's solution containing neutral red. (3) Unsealed preparations of connective tissue in plain Locke's solution. (4) Sealed preparations in Locke's solution. (5) Preparations of tissue from dead chick embryos. (6) Embryonic human blood in Locke's solution containing neutral red. (7) Adult human blood in neutral red solution.

Experimental Findings

All of the experiments, except in the dead tissue preparations, demonstrated the accumulation of neutral red granules and vacuoles in the cytoplasm of the fibroblasts during the degeneration stages of cytomorphosis. Microscopic examination revealed two distinct pictures, a negative, (*i. e.,* when no neutral red bodies were present) or nearly negative one, succeeded invariably by a positive phase (*i. e.,* by the presence of numerous neutral red bodies), becoming more pronounced as degeneration ensued.

Series 1.—The unsealed preparations in neutral red belong to a series of experiments in which none of the fibroblasts contained stained bodies when first examined—*i. e.,* within ten minutes after the application of the stain. After fifteen minutes a few scattered pink granules appeared in these cells and the tissue sometimes assumed a very faint pink coloration. These bodies rapidly increased in number, size, and intensity of color. From scarcely visible pink granules some of them became converted into angular, geometric forms of dark brick-red color, while others became larger in size and more rounded in outline, forming what are known as *vacuoles.* These bodies were paler and a more yellow-red than the granules. Frequently one or more of the brick-red granules

were found within the vacuoles, or in some instances the granules were surrounded by a pale yellow halo, which increased in size and became a vacuole. The granules ranged in size from scarcely visible specks to structures half a micron in diameter, while the vacuoles measured from five to ten times the diameter of the smallest granule. The cells remained alive and the neutral red bodies continued to accumulate in the cytoplasm for two hours or more. When the preparations were kept at a temperature of 39° C., there was actual propulsion of both granules and vacuoles through the cytoplasm. They appeared to travel along somewhat definite protoplasmic channels between the nucleus or the centrosphere and the more peripheral portion of the cytoplasm. The granules within the vacuoles exhibited Brownian movement.

The cytoplasmic arrangement of the granules and vacuoles was interesting and worthy of note. Their initial distribution was not constant; sometimes they were scattered uniformly throughout the cytoplasm, at other times they were gathered into clumps; again, the vacuoles and larger granules were grouped concentrically about the centrosphere and sparsely scattered at the periphery of the cytoplasm. Their final distribution, on the other hand, was strikingly characteristic, the granules and vacuoles being arranged about the centrosphere, as described by Lewis (1919).

Series 2.—In these sealed preparations, as in the unsealed, the granules and vacuoles appeared in about 20 to 30 minutes, increased in number and size but seemed to reach a maximum or standstill in about 45 minutes, the tissue dying shortly afterward.

Series 3 and 4.—The statements already made concerning the accumulation, size and arrangement of granules and vacuoles in the tissue prepared in solutions containing neutral red apply equally well to the cells of preparations in solutions without the dye. Except for a few highly refractive fat globules, mitochondria, and other granules, the cytoplasm of the fibroblast presented upon early examination a uniform appearance, which was later transmuted into a vacuolized structure having a lacy or frothy appearance. When Locke's solution containing neutral red was placed upon such a preparation, these vacuoles and granules immediately became stained in the same manner as did those which accumulated in the cells of the tissue placed directly in solutions containing neutral red, so that the two types of preparation could not be differentiated. The occurrence of vacuoles and granules in the unstained material is conclusive evidence that the phenomenon is not merely an accumulation of dye in the cell, but that there actually forms within the cytoplasm a structure which has a special affinity for neutral red.

Series 5.—The dead embryonic tissue in a solution of neutral red did not exhibit the intracellular accumulation of stained bodies but became diffusely colored, indicating that a marked change in the nature of the cell had taken place. Evans and Schulemann (1914) have demonstrated that dead tissue behaves quite differently from living cells, in that the benzidine dyes produce a uniform stain in dead cells. This may be due to a disintegration of the semi-permeable membrane, as McClendon and Mitchell (1911) have shown for other toxic substances. The uniform staining with neutral red is especially marked in tissue from embryos which have been dead for some time (undergoing autolysis) or in cell debris.

Tissue killed by fixation was found to behave quite differently. In some preparations the stain remained for some time, the results depending upon the dye employed and the fixing solution used. Lewis and Lewis (1915) stated that when the nile blue was used to stain cells which were later fixed, it disappeared from the vacuoles, and the mitochondria and fat globules then became stained. This did not occur in my observations when neutral red was used.

Dying cells showed all the transitions between the living cell and the cell debris. Contrary to the statement of MacCallum (1916, p. 53) that "a cell which had just died would look quite like its living neighbor," a moribund cell was easily distinguished from the living cells. The mitochondria became abnormal and disappeared; the cytoplasm became more fluid as was shown by the Brownian motion of the granules; and both cytoplasm and nucleus became slightly stained. Later the wall occasionally broke down and the cell became more deeply stained. At no stage in this degeneration did the neutral red leave the vacuoles to stain the mitochondria as claimed by Shipley, and when it did leave the stained bodies, the mitochondria had mostly disintegrated, and were therefore not present to become stained.

Series 6.—The blood of a human fœtus of six months, which I had an opportunity of examining through the courtesy of Dr. Shipley, did not exhibit any neutral red bodies except a few that developed in certain of the normoblasts.

Series 7.—A number of preparations of normal adult human blood were made in Locke's solution containing neutral red. When first examined, none of the blood cells except the eosinophiles contained any bodies stained with neutral red. The coarse granules, characteristic of these cells, stained at once a deep pink and later (within 10 to 40 minutes) the typical red vacuoles appeared (Fig. 5). Within 10 minutes after the application of the stain the specific granules of the polymorphonuclear leucocytes assumed a faint pink color. The vacuoles appeared within 15 to 30 minutes (Fig. 6) and were not unlike those in the fibroblasts and erythrocytes of the chick embryo. They were brick red and increased in size until much larger than those of the chick fibroblast. Vacuoles of different sizes also accumulated in the lymphocytes (Fig. 4), where they collected around the nucleus in such a way as partially to outline it.

The initial appearance of the vacuoles in the leucocytes was found to be partly dependent on the temperature at which the preparation is kept. When the blood smears were examined at 39° C. the vacuoles appeared earlier, the size increased more rapidly, and the bodies became larger than in those examined at room temperature. Movement of the vacuoles was more marked in the warm environment.

While the erythrocytes did not contain any neutral red bodies, when brilliant cresyl blue was used in the same manner as neutral red a blue body was observed in about 1 per cent of the cells.

In most of the preparations of chick material neutral red bodies were observed not only in the fibroblasts and clasmatocytes, but also in the erythrocytes (Fig. 3). In the last cells only vacuoles appeared. They were less numerous than in the fibroblasts, usually only one or two to a cell, although sometimes as many as ten were observed, in which case they outlined the nucleus. Stained vacuoles were observed in the erythrocytes of several preparations taken from a dead embryo in which the fibroblasts were apparently dead, as they stained diffusely.

The order of sequence in the development of the granules and vascuoles has not been conclusively determined. I am inclined to believe that in the fibroblasts the granular structure precedes the phenomenon of vacuolization, because in the tissues prepared in neutral red the granules are the first to appear. However, in the red blood cells and clasmatocytes of the chick, and in human white blood cells, as shown below, vacuoles appeared without being preceded by granules. This is contrary to the views held by Maximow (1916) and Shipley (1919), namely, that the vacuoles represent the final stage in the transition. In none of the preparations did the mitochondria become stained with neutral red, as has been claimed by Shipley (1915), Gatenby (1919) and others. The fact that in the eosinophiles the specific granules take the neutral red stain suggests the possibility that a neutral red granule may be normal in certain cells. In fact I have observed such granules in the amnion. These, however, were different in appearance and staining reaction from the bodies described above.

Discussion

The occurrence of bodies stained with neutral red, trypan blue, pyrrol blue, and other vital dyes, in the cytoplasm of the living cells has given rise to considerable discussion. Various theories have been formulated to explain the action of these stains; for example, the chemical theories proposed by Ehrlich and Overton and modified by subsequent investigators, and the physical, phagocytic theory advanced by Evans and Schulemann and supported by Clark and Clark, Matsumoto, Shipley and others. Then, too, diverse opinions have arisen concerning the nature of the granules, their relation to other cytoplasmic structures, and their physiological significance.

Action of the Stain.—The various theories regarding the action of the so-called vital dyes have been formulated largely by investigators who have used dyes other than janus green and neutral red. As my experiments were limited to these two stains, it is hardly practicable for me to discuss these theories at this time. However, in regard to the manner in which these dyes make their appearance in the cells, it seems to me that it is rather far-fetched to attempt to show a relation between this phenomenon and that of phagocytosis; for, regardless of how the stain makes its way into the cell, my experiments show that there is formed within the cell some bodies which have a special affinity for the dye—the mitochondria for the janus green and the granules and vacuoles for the neutral red. Without the presence of these bodies the dye does not become evident in the cell. It enters such a body and becomes accumulated therein in much stronger concentration than it was in the solution placed upon the cell.

Certain observers who have used neutral red have emphasized the fact that the death of the cell causes the neutral red to leave the stained bodies and appear as a diffuse stain in the cytoplasm. It is difficult to understand how the death of the cell could cause any ingested substance to leave a vacuole and become diffused through the cytoplasm unless it had been held in some chemical combination within the vacuole.

Nature of the Neutral Red Bodies.—Plato (1900), in conclusions drawn from experiments in which leucocytes were fed with red blood cells, dried white of egg, spermatozoa, and other cellular products, claimed that the bodies which appear in the cell under these conditions and which stain with neutral red are albuminous in nature. Galeotti (see Plato, 1900, p. 869) concluded that these bodies are food substances or secretion products. Cesa-Bianchi (1910) observed neutral red granules in the central half of the renal cells of the white mouse, and believed them to be liposomes because they stained with neutral red.

Albrecht (1903) found that liposomes of fresh muscle did not stain with neutral red, but that if kept aseptic at 37° C. in normal salt solution they took up the dye.

Bell (1911, p. 297) dissented from this view, as he did not succeed in staining any granules in muscle cells with neutral red. He stated: " In the kidney it is easy to show large numbers of neutral red granules. The number, size and arrangement of the granules show that they cannot correspond to the liposomes. The liposomes are not stained."

It has been suggested by McClenden and Mitchell (1911) that the neutral red bodies are lipoid in nature. According to them the egg is filled with lipoid particles which take neutral red. The view that they are lipoid in nature has not passed without opposition (Harvey, Aschoff and others). Aschoff (1910) found that none of the lipoid substances (lipochromes, neutral fats, free fatty acids, soaps, or cholesterin) stained with neutral red, except the myeline phosphatid (cerebroside) in autolysis. Harvey (1913) also questions the lipoid nature of the granules and states that the latter are probably lecithoproteid in nature. According to Mathews (1916), if an emulsion is made of lecithin or other phosphotide, egg albumen, and olive oil, it will be found that the granules of lecithin or phospholipin take up neutral red. Although he shows that other colloids of an electro-negative or anionic character stain in the same way, the behavior of some of the small granules of the cell in ether and when centrifuged seems to him to indicate that they are very much like lecithin.

Relation of Neutral Red Bodies to Mitochondria.—Cowdry (1914), Lewis and Lewis (1915), Scott (1916), Sundwall (1917), M. R. Lewis (1917), and W. H. Lewis (1919), who

have used the double stain, have demonstrated that the mitochondria in connective-tissue and other cells stain with janus green in the presence of neutral red granules; and they have found no relation between the mitochondria and the neutral red bodies.

Duesberg (1911) mentioned various writers who claimed that certain vital dyes stain the mitochondria, but he himself doubted that neutral red and methylene blue possess this property.

Shipley (1915) first claimed that the mitochondria stain with neutral red, but later (1919) arrived at the conclusion that the mitochondria are not colored by neutral red but are specifically stained with janus green.

Gatenby (1918), in a communication on the cytoplasmic inclusions of the germ cell, stated that the mitochondria are stained *supra vitam* by janus green, neutral red, etc. Since it has been shown that neutral red does not stain the mitochondria, it is obvious that Gatenby was dealing with two types of granules, one of which takes neutral red, the other janus green, as was shown for the germ cells of the grasshopper by Lewis and Robertson (1916).

It appears to me that there are in general four distinct types of cytoplasmic granulations; the mitochondria, which stain with janus green; granules and vacuoles that stain with neutral red, the specific granulations normally present in the cell (for instance, the granules in the leucocytes and the eosinophiles); and fat globules that are not stained by either neutral red or janus green.

Physiological Significance of the Neutral Red Bodies.—In regard to the physiological significance of the neutral red bodies the observations given in the literature would seem to deal with three groups: (1) Structures which form in degenerating tissue, (2) digestive vacuoles, (3) bodies phagocytized by the cells.

Many of the investigators conclude that these bodies are abnormal or are an indication of the degeneration of the cells. W. H. Lewis (1919), in a detailed study of the significance of neutral red bodies in tissue culture of chick connective tissue, found that, as the cells grew old and degenerated, vacuoles and granules accumulated around the centrosphere. Both the vacuoles and granules became stained when a solution of neutral red was placed upon the culture. Owing to the fact that the accumulation of the granules and vacuoles was associated with the degeneration or approaching death of the culture, Dr. Lewis called the bodies degeneration vacuoles and granules.

Arnold (1899, p. 426) fed polymorphonucleated blood cells with neutral red. After a short time granules appeared. These soon exhibited variations in size and were sometimes inclosed in vacuoles. Referring to the vital staining of the tissues of plants and lower animals, Arnold states (p. 431) that the majority of authors have reached the conclusion that staining of cells takes place first when these cells begin to degenerate.

Minot (1912) described three ways in which the disintegration of erythrocytes of human embryos may take place: *i. e.*, by fragmentation, by bursting of the corpuscle, or by vacuolization; and he was perhaps the first to mention vacuoles in blood cells as an evidence of cell injury. Emmel (1914), who studied erythrocytes in the pig embryo, also considered the regressive and degenerative changes of the cells. He thought that the erythrocytes, taken from the circulation, must be in various stages of normal physiological degeneration and disintegration, and regarded the appearance of vacuoles in the cytoplasm as a degenerative change, a change which is most marked "in old cultures, homoplastic cultures, and in those experiments in which the plasma had been modified."

Cowdry (1914) observed, in the cytoplasm of finely granular leucocytes (neutrophiles), bodies that were stainable with both cresyl blue and neutral red. He stated: "It seemed clear that I was dealing with an accumulation of material resulting from the interaction of the stain and the cytoplasm, not with formed bodies preexistent in the living, unstained leucocytes." As claimed by Cowdry, the body which took the stain was not present in the normal cell, but neither was it caused by "the interaction of the stain and the cytoplasm." It is caused rather by an alteration of the cell itself during observation.

Lewis and Lewis (1915), in their observations on the mitochondria in tissue cultures, noted the formation of vacuoles during the degeneration of certain of the older cultures and attributed it to an excess of waste products accumulated around the cell.

Shipley (1916) noted the appearance of vacuole-like bodies which he thought were an "index of a degeneration change of some sort," because they were more numerous in trypanosomes which were losing their motility.

MacCallum (1918, p. 88) regarded the granules as degeneration products associated with pathological changes in the cell. The proteid nature of the granules occurring in the pathological condition known as "cloudy swelling" he seemed to consider as established, but he raised the question as to the origin of these granules and their relation to other structures of the cell.

Renaut (1907, p. 509), studying sealed preparations of the connective tissue of an adult rabbit in solutions of neutral red, found that the fibroblasts became full of vacuoles containing granules. In the embryonic connective tissue of sheep and cattle the appearance of the neutral red bodies was slower. They gradually developed, however, and were numerous at the end of half an hour. While Renaut did not perceive that they were abnormal, it seems to me quite probable that the neutral red bodies described by him, and also the so-called "liposomes" of Albrecht, are not normal structures of the cell but are comparable to those observed in my experiments, since the methods employed by these observers were sufficiently similar to my own to have produced abnormal structures.

Degeneration, as manifested by the appearance of vacuoles, has been frequently referred to in the literature; and, although in some cases neutral red was not used, it appears to me that certain of the structures observed are the same as the above described degeneration vacuoles which take the neutral red. Lillie (1908, p. 317) wrote: "Vacuolization of the tissue be-

tween the cloacal membrane and the urodæum indicates its subsequent disappearance."

Calkins (1909, p. 81) stated that, while numerous "gastric vacuoles are present in the normal and active paramecium, they disappear when starved, and that "great vacuoles" appear which increase in size with starvation.

Nickerson (1913), studying the intercellular canals in the skin of Phascolosoma, similarly noted the appearance of large vacuoles in old and degenerating cells, as well as differences in the staining properties of young and old cells.

On the other hand, a number of observers have claimed that the bodies which take the neutral red are not degeneration products but are digestive vacuoles. The distinction, however, should be easily made, since neutral red is itself an indicator (Harvey 1913), and many writers have asserted that the digestive vacuole is slightly acid (cerise red with neutral red). Metchnikoff (1907, p. 11) mentioned the presence of digestive vacuoles in the endoplasm of Paramecium and Infusoria in general, which feed upon numerous bacteria, and stated that the fluid content of these vacuoles has a "distinctly acid reaction," as demonstrated by the use of neutral red. Policard (1910) noted a difference between the brick-red degeneration vacuoles and the "cerise red" food vacuoles. Calkins, discussing the nature of the digestion processes in protozoa, emphasized the work of Englemann and Le Danteux, as well as that of Metchnikoff and others who showed acid reaction in gastric vacuoles of certain protozoa.

I have had occasion to observe amœbae stained with neutral red and have been immediately impressed with the decided rose tint of digestive vacuoles as compared with the yellow or brick-red color of those in my present observations. The fact that digestive vacuoles stain with neutral red may account for the discrepancies that have arisen concerning the functional activity of the degeneration vacuoles. Prowaczek (see Plato, 1900) was one of the first to deny that neutral red bodies in most cells were food vacuoles, and he believed them to be digestion granules or ferment bearers.

A number of writers have asserted that appearance of the neutral red bodies in the cell is related to the phenomenon of phagocytosis as described by earlier observers (Plato 1900, MacCallum 1903, Metchnikoff 1907). Plato claimed that the material which takes the stain is phagocytized by the cell. Clark and Clark (1918) and Matsumoto (1918) concluded that the dye itself may be phagocytized, while Shipley (1919) favored the theory of phagocytosis of substances which form the vacuoles, appear within a vacuole, or stain the vacuole. It seems to me hardly probable that cells in a simple salt solution such as Locke's solution, from which the oxygen was largely excluded, could have phagocytosed the material which formed the vacuoles and granules. On the other hand, the disturbed metabolism of these cells might result in the accumulation within the cytoplasm of substances which normally would have been eliminated.

Conclusions

1. Bodies having an affinity for neutral red accumulate in the cytoplasm of fibroblasts and erythrocytes of chick embryos when observed in film preparations.

2. Neutral red bodies are not present in dead tissue but are found in many kinds of degenerating cells.

3. The granules and vacuoles which appeared in cells studied in this manner were not due to the accumulation of dye in the cytoplasm nor to an interaction of the dye and the cytoplasm, but were the results of the abnormal condition of the cell.

4. The white cells of human blood observed in film preparations also developed vacuoles which stained with neutral red.

5. These bodies are probably not phagocytized by the cell nor do they correspond to the food vacuoles.

6. The behavior of the cells in film preparations in Locke's solution led to the conclusion that there are present certain bodies (the mitochondria) which stain with janus green but not with neutral red, and others, varying in number according to the condition of the cell, which stain with neutral red; also, that neither of these types arises from the other. In addition to these, there may be granules specific for a given type of cell, and also fat globules.

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DESCRIPTION OF PLATE

Camera lucida drawings showing the arrangement of neutral red granules and vacuoles, mitochondria and fat globules in various cells described in the text. Neutral red granules are shown as black dots; vacuoles as shaded circles; fat globules as black circles; and mitochondria as black rods and threads.

FIG. 1.—Fibroblast of a chick embryo showing neutral red granules and mitochondria.

FIG. 2.—Fibroblast of a chick embryo in which some of the granules are enclosed in vacuoles.

FIG. 3.—Erythrocyte of chick embryo containing vacuoles only.

FIG. 4.—Lymphocyte of adult human blood, exhibiting vacuoles and specific granules.

FIG. 5.—Eosinophile of adult human blood showing large vacuole and specific granules.

FIG. 6.—Neutrophile of adult human blood containing several vacuoles and specific granules.

NOTES ON NEW BOOKS

Gynecology. By BROOKE M. ANSPACH, M. D., with an Introduction by Dr. John G. Clark. Cloth, $9.00. (*Philadelphia and London: Lippincott Company, 1921.*)

The adequate presentation of even the essentials of gynecology, as it is practised to-day, within the confines of a single volume text-book is a task of no mean proportions. For not only does it involve a many-sided consideration of the female reproductive organs but also and necessarily of certain phases of abdominal surgery, of orthopedic surgery, of obstetrics, of urology, of internal medicine and of physicotherapy.

No one, however, who is familiar with Dr. Anspach's work as student, investigator, teacher and practical surgeon would question for a moment his ability to perform such a task with enviable efficiency. And when one finds the finished product dedicated to and sponsored by Dr. John G. Clark, who for years has so brilliantly adorned the Philadelphia clinic and whose name is synonymous with all of the best traditions of our profession, the inevitable mental reaction is one of pleasant anticipation rather than an attitude of critical fault-finding.

Moreover, a careful perusal of this book reveals that it has an intrinsic and substantial worth to recommend it quite aside from the happy circumstance of gifted parentage.

The opening chapters are devoted to an unusually lucid, concise, yet comprehensive adaptation to gynecology of the relevant matter from embryology, the developmental anomalies, anatomy and physiology. Next follows a series of chapters setting forth the details of history-taking and methods of examination, including not only the general physical as well as those special procedures available for investigating the pelvis and abdomen, but also the technique of a complete urologic study as well as that required for accurate inspection of the anus and rectum.

The diseases of each unit of the reproductive and urinary systems are then presented on an anatomical basis and discussed in a thoroughly satisfactory and logical manner.

In addition, the author has wisely incorporated a special chapter dealing with those diseases of the abdominal viscera so commonly associated with pelvic disorders and constantly requiring proper handling at the operating table.

Other special chapters are devoted to gonorrhœa, syphilis, and tuberculosis of the pelvic organs; one to the important subject of backache; one to menstrual disorders; and another to sterility.

While it was obviously impossible to incorporate in a work of this size all of the available operative procedures, yet admirable selective judgment has been used in the choice of those recommended, and in not a few instances the details of several are given.

Instructive and suggestive chapters are given on the preparation of the patient for operation, operative technique, post-operative management and complications.

Especially noteworthy are the chapters on hygiene of adolescence, mechano-, sero-, thermo-, and radio-therapy.

Appended to each chapter is a bibliography which includes references to all of the classic as well as the more important collateral contributions to the particular subject dealt with. This is a most excellent feature of the book and conspicuously reflects the author's familiarity with the literature as well as his painstaking care and judicious skill in estimating the work of others.

On the whole, the illustrations are excellent, the publisher's work has been well done and the index is adequate.

In a word, rarely has a text-book of this size been produced which possessed so many excellent qualities with so few deficiencies as does this one of Dr. Anspach. It reflects great credit upon the author and his associates and can be recommended without reservation to students and to the profession at large. E. H. R.

The Basis of Psychiatry. By ALBERT C. BUCKLEY, M. D. (*Philadelphia and London: J. B. Lippincott Company, 1920.*)

Buckley's book is an effort to adapt the psychiatric teachings of the Philadelphia School to the biological point of view. This shows, on the one hand, in the great amount of elementary biology and histology that is introduced and, on the other, in the acceptance of the concept of reaction-types, which leads to such an unusual and suggestive occurrence as the formation of a special chapter on the "præcox-manic problem." In the actual discussion of the psychiatric material the acceptance of a biological viewpoint is very much less in evidence. The psychology and psychiatry remain very much in the traditional garb and substance, with liberal rehearsal of the older psychiatry and the German additions of the late nineties, but very little contact with the later additions of American workers. The book is a means of handing on tradition and neither stimulating for actual investigation nor suggesting evidence of being based on material of experience worked out with psycho-biological and biological methods. It does, however, represent very truthfully a good average type of present-day practical psychiatry—conservative as to psychobiology, but willing to borrow analogies at least from the fundamental biological viewpoints. The real step towards a frank recasting of the psychiatric material and problems themselves on a psychobiological basis still remains to be taken. A. M.

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THE THEORIES OF BLOOD COAGULATION[1]

By Jules Bordet

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First of all, I beg of you to excuse my imperfect knowledge of the English language and to accept my best thanks for the honor you have conferred upon me by inviting me to deliver the Herter lectures. I shall try today to give a brief *résumé* of the chief theories which have been held concerning the mechanism underlying the coagulation of the blood. This phenomenon deserves our interest not only because of its physiological importance but also as a striking example of the resources of experimental analysis. It can occur *in vitro,* and this is a very favorable condition for the success of investigation. Nevertheless, and although it has been the subject of innumerable researches, up to the present time, its mystery has not been completely disclosed. You will not expect me to attempt a detailed review of the whole subject. I shall give only such broad outlines as will serve to make clear the modern conceptions which seem to afford the best explanation of this complicated process, and which, therefore, especially deserve our attention.

[1] Lecture I of the Herter Series, delivered before The Johns Hopkins University on Tuesday, October 26, 1920.

It is hardly necessary to remind you that coagulation is nothing else than the aggregation, into meshes of fibrin, of particles of fibrinogen, a substance which, as Fredericq showed 43 years ago, preexists as a dispersed colloid in the circulating plasma. When the blood flows from a wound, the first determining factor which, through successive modifications of the plasma, assures the solidification of fibrinogen, is not infrequently the mixture of the blood with very active principles liberated by the bruised tissues, or in other words, the addition to the blood of tissue extract. But such an influence is an additional one, foreign to the blood itself; hence, limiting the problem, I shall consider here, exclusively, the coagulation that the blood is capable of showing, solely by means of its own substances.

A most important, even a decisive factor of this automatic coagulation, is the contact of a foreign solid body (glass, for instance), which acts only physically by its presence, since it does not liberate any soluble substance. The contact, external factor, brings into activity the internal factor, belonging to the blood, and that is the process through which the principle

directly and immediately responsible for the coagulation, the fibrin-ferment or thrombin, is produced. In fact, the thrombin, which is found in large quantities in the clot or in the serum, does not exist (as Schmidt showed many years ago), in the circulating blood. Consequently, several stages are to be distinguished in the total process, the most important one being the period in which the thrombin appears, the fibrinogen itself playing merely a passive rôle. Fibrinogen can be extracted by special methods and obtained in a fairly pure condition, but it must be kept in mind that the essential problem presented to the physiologist is the coagulation, not of pure fibrinogen, but of the blood considered as a whole, that is, of a very complex medium, cellular and plasmatic, having a definite reaction and definite osmotic pressure, containing numerous constituents, and especially colloids, which presumably are apt to influence each other through molecular adhesion. Coagulation could not be studied without taking into consideration every influence apt to interfere in the phenomenon.

Since the blood of mammals, as a rule, clots promptly, it seemed essential to the success of the investigation to determine how the course of the process could be protracted and, moreover, how it could be stopped at the first period of its evolution, so as to make possible the separation of the cells from the still liquid plasma. Several methods have been devised; I shall mention them very briefly.

Concentrated salts, magnesium sulphate for instance, hinder the coagulation. Common salt especially answers the purpose, being a normal constituent of the organism. Blood from the artery to which from three to five per cent of salt has immediately been added yields by centrifugalization a clear plasma which does not clot so long as the high saline concentration is maintained, but which, when diluted with an amount of distilled water sufficient to reestablish the normal saline content, clots rather quickly. Decalcifying salts, the type of which is sodium oxalate, prevent completely the coagulation, calcium salts being necessary to this phenomenon. By centrifugalization a clear plasma is obtained, which tends to clot when a soluble calcium salt, such as calcium chloride, is restored.

Coagulation is also prevented if to the active contact of glass a contact is substituted, which is not, if we may so express it, felt by the blood or the plasma. A liquid does not feel a wall, I mean does not react physically to it, unless it is capable of adhering to it. Freund was the first to show that blood flowing from the artery does not clot, or at least clots very slowly, when received in a vessel the inside of which has been coated with oil or vaseline. Since it forms a solid coating, paraffin is very suitable to such experiments, and frequently permits the separation of the cells and the plasma by centrifugalization. In conjunction with Gengou I observed that by these means a clear plasma can be obtained and be kept fluid for 24 hours, but that clotting soon occurs when the plasma is brought into contact with glass. This experiment shows that the contact of glass can bring about its effect without the presence of any cells, that is, without any vital interference—we have to do with a physico-chemical phenomenon.

The blood of certain animals, namely, birds and fishes, as Delezenne has shown, clots very slowly by itself, coagulation being greatly hastened by the addition even of traces of tissue extract. Without the help of decalcification or of paraffin coating the blood of a bird will remain fluid for a long time and even yield by centrifugalization a permanently liquid plasma, provided that the tube inserted into the vessel has not been allowed to touch the wound, and consequently no trace of tissue extract has become mixed with the blood. As a matter of fact, this precaution ought to be taken regularly, whatever may be the species of animal under experiment, as it is quite a general rule that tissue extract accelerates coagulation, this auxiliary influence being particularly noted for birds, because this blood is not so capable of spontaneous coagulation as is that of mammals.

Thanks to such methods, the separation of the two constituents, cells and plasma, can be attained before any beginning of coagulation and—let us emphatically insist upon this essential fact—before any appearance of the coagulating principle, thrombin. There is no need to remind you that serum yielded by coagulation contains thrombin.

We must now try to go further and subject plasma and cells to a closer analysis. Let us consider first the plasma. Soluble calcium salts are necessary to coagulation. How do they act? Pekelharing and Hammarsten have shown the essential fact that these salts are not necessary to the transformation of fibrinogen into fibrin under the influence of thrombin, but are indispensable to the formation of the latter, that is, to the production of thrombin from the mother-substances already present in the circulating blood. The production of thrombin is prevented by oxalate, but on the other hand decalcification does not prevent the coagulation of the fibrinogen by completed thrombin. Indeed, it has been proved that blood, oxalated immediately after withdrawal from the artery, remains permanently fluid, no thrombin being ever detected in it, whereas if serum yielded by normally clotted blood be oxalated, this oxalated serum, added to oxalated plasma, causes the coagulation of the latter. From these facts it follows that oxalated plasma is a most suitable reagent for the detection of thrombin in a given liquid; indeed, an estimation of the coagulating power of such a liquid may be made by taking into consideration the quantity of oxalated plasma coagulated by a certain amount of this liquid, or the rapidity of the occurring coagulation. But it must be borne in mind that, at least when present in serum, the activity of a given thrombin depends not only upon its quantity but also upon its age. The capacity of fresh serum to coagulate oxalated plasma decreases very quickly, by a spontaneous attenuation of the thrombin, and this fact affords a possibility of detecting whether a given thrombin has been produced quite recently or some time ago. Several experiments, as we shall see, require such a determination.

Contact is also necessary to coagulation. How does it operate? I showed with Gengou, many years ago, that con-

tact is in a way analogous to calcium, or in other words, that contact with a foreign solid body (paraffin of course excepted) is necessary to the appearance of thrombin, but is not requisite for the coagulating influence of the latter. When blood is received into a paraffined vessel, thrombin is not formed; when received into a glass vessel, thrombin is produced in the zone of contact, a fact which explains why coagulation begins along the wall. But when serum yielded by previously clotted plasma is added to blood or plasma kept in a paraffined vessel, the entire mass rapidly solidifies, the paraffin no longer exerting any inhibiting influence. This experiment explains why blood freshly drawn and placed in a glass vessel coagulates in a mass much more rapidly when shaken.

What then is the origin of thrombin? It does not exist as such in the circulating blood, although the latter contains everything requisite for its production. The circulating blood, therefore, contains the mother substance, or mother-substances, of thrombin, which, for convenience, may be called prothrombin, and which in the early stages of coagulation is converted into thrombin. What is prothrombin?

Sixteen years ago Morawitz made an important discovery in this connection. He found that if crushed tissue, muscle for example, is added to serum yielded by normal coagulation, the coagulating power of this serum towards oxalated plasma is considerably increased. And yet, the extract of tissue by itself does not contain any thrombin, since without the help of serum, it is incapable of coagulating oxalated plasma. Hence, we are forced to conclude that the tissue extract contains something which is not thrombin, but which reacts with the serum so as to produce this active principle. Thence follows at once the hypothesis that thrombin is derived from the interaction of two different substances, the one furnished by the tissue cells, the other by the serum. Undoubtedly, even before the introduction of the tissue extract, a certain amount of thrombin existed in the serum, but it seems as if this fluid contained also an excess of the mother-substance, the latter being capable of reacting with the tissue extract so as to generate a fresh supply of thrombin.

But the question immediately arises whether such an assumption, deduced from experiments in which tissue extract plays an important rôle, may be without further question applied to the automatic coagulation of pure blood. As a matter of fact, it must be kept in mind that injected into the circulation, the tissue extracts are highly toxic, causing sudden death due to intravascular coagulation. Undoubtedly, they contain some coagulating principle foreign to the blood itself. This substance upon which I shall not dwell today, our present task being merely the study of the coagulation of pure blood, in all probability, is of an albuminoid nature, and is markedly thermolabile. It is specifically related to the tissues, does not exist in the blood, and could not be considered as a real mother-substance of thrombin. But we must immediately add that, besides this peculiar principle, the tissue cells, nevertheless, contain one of the two mother-substances of thrombin. This exists also in the blood cells, is of lipoid nature and may be designated *cytozyme*. The other mother-substance, called by us *serozyme*, is furnished by the blood fluid and is present in the serum. But let us see how these determinations have been arrived at?

The assumption that the blood cells furnish one of the mother-substances of thrombin is in perfect accordance with the results of experiments dealing with the part played by those cells, and chiefly by platelets, in the coagulation. Platelets can be easily separated by centrifugalizing oxalated blood at a moderate speed for a short while; being very light, they remain in suspension, whereas red and white corpuscles are thrown down, the turbid supernatant fluid pipetted off being very rich in platelets. Now if such a platelet-plasma is centrifugalized for a long time and at a very high speed, the platelets finally are sedimented and a clear plasma may be obtained from which the platelets have not been thoroughly eliminated, this being impossible, but in which they are present only in small numbers. Comparing these two plasmas, the one very rich, the other very poor in platelets, Lesourd and Pagniez found that after recalcification the former clots rapidly, the latter slowly. Completing these experiments, with Delange, by comparing the coagulating influence, on oxalated plasma, of the two serums yielded respectively by the coagulation of plasma rich in platelets and of plasma poor in platelets, we observed that the former serum contains a much larger quantity of thrombin than the latter. Consequently, the platelets actively participate in the production of the coagulating principle. This fact can be proved more distinctly by the following experiment: A sediment constituted exclusively of these small cells is obtained by vigorously centrifugalizing oxalated plasma, previously freed of its red and white corpuscles, but containing still its platelets. This platelet deposit, thoroughly washed, is emulsified in physiological solution, and one drop of the turbid emulsion thus obtained is added to a certain quantity of a serum which, being derived from the slow coagulation of recalcified exalated plasma that has been freed of its own platelets, is by itself very poor in thrombin. Within 20 or 30 seconds, the mixture becomes capable of coagulating a suitable amount of oxalated plasma almost instantaneously; in other words the reaction of serum with platelets generates an abundant supply of thrombin. It must be pointed out that this experiment closely resembles that of Morawitz except that platelets instead of tissue cells are added to the serum. Tissue cells and platelets both contain one of the generators of thrombin, which may be called *cytozyme*. The second one, the *serozyme,* exists in the serum. It can be easily demonstrated that the reaction between serum and platelets, that is to say, between serozyme and cytozyme, takes place only in the presence of soluble calcium salts, no thrombin appearing if the serum has been decalcified before the introduction of the platelets. Moreover, I shall further insist on the fact that the two substances unite, that they really consummate each other. Indeed, experience shows that when a serum has been treated once with platelets, and has yielded thrombin, this serum is subsequently incapable of reacting with a new amount of platelets, its serozyme having been exhausted by the first reaction. It follows that a serum pro-

duced by the coagulation of a plasma rich in platelets and which of course contains much thrombin, is considerably less rich in serozyme, that is, considerably less capable of reacting with new platelets, than is a serum derived from a plasma deprived of most of its platelets. This is precisely what experience shows. It is, therefore, highly advisable always to employ for serozyme a serum obtained by the coagulation of oxalated plasma which has been carefully freed of its platelets before recalcification.

Serozyme is a thermolabile substance, easily destroyed by heat. No thrombin is produced when platelets are added to serum that has been exposed to a temperature of about 56° C. On the contrary, cytozyme, the active principle of platelets, may be heated to a hundred degrees and even higher without losing its properties; cytozyme is thermostable and, furthermore, can be easily extracted.

A thick emulsion of platelets, treated with a large excess of absolute alcohol, gives an extract from which, by evaporation, a residue is obtained which is soluble in alcohol, ether, toluol and chloroform but insoluble in acetone, thus exhibiting the characteristics of lipoids, and especially of lecithin. This residue acts as a very powerful cytozyme.

As we were able to show eight years ago, traces of this lipoid behave exactly as do platelets, generating thrombin when added to serum, hastening the coagulation of recalcified oxalated plasma or causing the coagulation of spontaneously non-coagulable bird's plasma. The same lipoid, possessing exactly the same properties may be extracted from tissues, from muscle for example. Cytozyme is thus a lipoid.

These facts having been obtained about cytozyme, what is serozyme? Serozyme is certainly furnished by the plasma, not by the cells. Platelets contain cytozyme; they yield thrombin when mixed with serum but they are never able to liberate thrombin when kept in physiological solution or in distilled water, even in the presence of calcium salts. They consequently contain only one of the mother-substances, not both of them. The lability of serozyme towards heat allows us to presume that this substance is of an albuminous nature. Its fragility would be a very serious hindrance to its isolation, but for one of its properties—a really fortunately one. The serozyme shows a strong tendency to adhere to mineral precipitates, barium sulphate or calcium fluoride for example. This is the reason why, as I discovered many years ago with Gengou, those precipitates, added to oxalated plasma, wholly suppress in the latter the property of coagulating by subsequent recalcification: one of the mother-substances, the serozyme, which is absolutely requisite for the production of thrombin and consequently for coagulation, has been entirely removed. I have found more recently, in conjunction with Delange, that tricalcic phosphate is especially powerful as an absorbent. When diluted in physiological solution, this substance gives a rather gelatinous emulsion, a very slight quantity of which, added to blood flowing from the artery, prevents its coagulation. By centrifugalization and pipetting off, a clear plasma is obtained, which remains fluid, even when platelet emulsion, or tissue extract, or lipoidic cytozyme, is added. This is easily understood; both mother-substances, serozyme and cytozyme, are equally necessary to the production of thrombin; it is of no use to add one of them if the other is absent. But such a plasma, which we may for the sake of brevity call "phosphate plasma," clots under the influence of thrombin or, which naturally is the same, when both mother-substances are added. It behaves as an excellent reagent for the detection of thrombin. Since its composition closely resembles that of the original plasma, it may be considered as being fibrinogen dissolved in a normal medium.

But tricalcic prosphate is endowed with a property which is remarkably useful for technical purposes. As is well known, it is capable of being dissolved in physiological solution under the influence of a current of carbonic oxide gas. Consequently, phosphate which has been added to plasma and has absorbed the serozyme, after having been thoroughly washed, can liberate, thanks to its own dissolution, the active principle it had withdrawn. In this way, Delange and myself succeeded in bringing about the isolation of serozyme, which, on the addition of cytozyme extracted from platelets, gave abundant thrombin. Thus in the course of the whole experiment the factors which determine coagulation are in reality subjected to an analysis followed by a synthesis.

As mentioned above, our assumption that serozyme and cytozyme are the generators of thrombin involves the idea that those mother-substances really unite to form a compound, which is thrombin. This ought to be demonstrated also with regard to pure cytozyme, I mean a cytozyme in the condition of a lipoidic extracted matter. If the union really occurs, we may anticipate that, if a given quantity of serozyme be mixed with a sufficient amount of cytozyme, thrombin will be engendered and the serozyme will be exhausted; in other words, it will be no longer capable of yielding fresh thrombin when a new amount of cytozyme is added. Such is indeed the case. Serum yielded by coagulation of recalcified platelet-free oxalated plasma is divided into two parts, lipoidic cytozyme being added to one of them, the other portion being kept as it is. In the tube containing both serum and cytozyme, thrombin appears, the activity of which is very strong at the outset, but decreases, as we know, very rapidly so as to become quite attenuated by the following day. On the next day, lipoidic cytozyme, and, several minutes afterwards, oxalated plasma are added to both tubes. Then the tube which has received cytozyme on the preceding day does not clot at all, or does so only very slowly, whereas the tube, to which cytozyme has just been added for the first time, clots almost instantaneously.

It is clear that such an experiment makes it possible to ascertain whether the same cytozyme, endowed with the same binding properties as the pure lipoid, exists in fresh or heated platelets, or in tissue juice, ground muscle for example. Adequate experiments show that serum which has already reacted with any one of such materials does not generate any more thrombin when subsequently brought into contact with any one of them. For example, serum to which lipoidic cytozyme has been added will not react any more either with the same lipoid, with platelets, or with muscle juice, and conversely.

Without entering into details, I may add that the manner in which the two substances unite closely resembles the mode of union of toxins and antitoxins, in that the process is not governed by the law of strict and constant equivalents, but takes place in varying proportions, thus seeming to result not from true chemical affinities but from contact affinity or molecular adhesion. But another fact, more noteworthy for its bearing upon the underlying mechanism of coagulation is disclosed by the determination of the lapse of time required for the union of both substances.

Serozyme being found in serum may be assumed to exist also in the oxalated plasma from which this serum has been derived. Now if cytozyme and serum are mixed, thrombin appears very quickly; it is only a question of some seconds. But—and this fact is undoubtedly remarkable—if cytozyme be added, not to serum yielded by coagulation of recalcified oxalated plasma, but to an identical oxalated plasma recalcified just before, that is, at a moment when this plasma is still perfectly fluid, the appearance of thrombin is greatly delayed. In other words, serozyme reacts with cytozyme quickly when present in serum, slowly when present in plasma. We thus reach the conclusion that the serozyme does not exist in the same condition in plasma as in serum, that in plasma it is not capable of reacting at once with cytozyme. We may express this fact by saying that plasma contains proserozyme instead of active serozyme, one of the first phenomena of the whole process of coagulation being precisely the conversion of proserozyme, unfit until transformed to unite with cytozyme, into serozyme capable of this reaction.

The notion that in original plasma or in circulating blood, serozyme does not exist as such, that is, does not exhibit affinities toward cytozyme, satisfactorily explains why intravascular injections of the latter substance are, as we found, quite harmless. But the blood of such injected animals shows, when shed within about half an hour after the injection, a strikingly increased tendency to rapid coagulation. This fact as we have pointed out may probably be available for therapeutic purposes in cases of hemorrhage.

Is it now possible to investigate under what influences the proserozyme is converted into serozyme, in other words, under what influences does it acquire the capacity of reacting with cytozyme?

To solve this problem, we have at our disposal a very adequate technic, based on the use of oxalated salt-saturated plasma.

Some minutes ago I mentioned the fact that when oxalated plasma is saturated with common salt, the fibrinogen is entirely precipitated. After strong centrifugalization, pipetting off and elimination of the excess of salt by dialysis in presence of physiological oxalated solution, the supernatant fluid represents exactly normal oxalated plasma, except that, having lost all of its fibrinogen, it cannot coagulate. Being oxalated it does not contain any trace of thrombin, but is still capable of producing plenty of it on the addition of calcium salt and cytozyme. Now if a calcium salt and cytozyme are added thrombin appears indeed, but only after a rather important delay. Half an hour, and sometimes more, must elapse before the mixture becomes capable of bringing about an almost instantaneous coagulation of a fibrinogen solution. Thus the acquisition of the ability to react with cytozyme, that is, the conversion of proserozyme into serozyme, requires a notable length of time. Now, on the other hand, if the aforesaid fluid without fibrinogen is recalcified and cytozyme is added one or two hours later the thrombin appears almost instantaneously. This experiment clearly illustrates the essential assumption that the whole process of the production of thrombin, the first stage included, which is the conversion of proserozyme into serozyme henceforth capable of uniting with cytozyme, takes its course without any participation of fibrinogen.

Furthermore, the conversion of proserozyme, which as we know cannot take place without calcium salts, is—and the fact is noteworthy—strikingly favored by contact with glass. The capacity of reacting with cytozyme appears only after a much longer lapse of time when the recalcified fluid is kept in a vessel coated with paraffin. Consequently, the influence of contact, which is so obvious in coagulation, is not exerted through some interference of fibrinogen, but really acts without any help of the latter, as a factor of thrombin production. It is highly probable that contact, by way of adsorption, frees the liquid of some antagonistic substance, most likely some protective colloid, which prevented the serozyme from reacting with cytozyme, that is, maintained it in the inactive condition of proserozyme. On the other hand, experiments show that the presence of cytozyme likewise facilitates such a liberation of serozyme, owing to its strong affinities towards the latter principle.

To sum up, we are now able to follow the schema which indicates the order of succession of the phenomena.

SCHEMA OF COAGULATION

Plasma		Cells (platelets)
fibrinogen	proserozyme converted into serozyme by means of calcium and contact along the wall.	cytozyme liberated by means of contact (adhesion of cells along the wall).

union of serozyme and cytozyme
↓
→ ←—thrombin (along the wall)
fibrin
(contact and calcium no longer necessary)

I think that the schema symbolizes quite accurately the most prominent features of the whole process and distinctly shows the sequence of events. But the mechanism underlying coagulation as it occurs under ordinary conditions is still somewhat more complicated, owing to a peculiar property of thrombin. Thrombin results from the union of serozyme and cytozyme, but these two substances combine in variable proportions. The consequence is that a given complex, when rich in serozyme, is able to capture an additional amount of cytozyme, and, when rich in cytozyme, which is ordinarily the case in the coagulation of whole blood, shows a marked affinity towards a new amount of serozyme. As a matter of fact, such an affinity

is so strong that it causes thrombin to attract and to possess itself of serozyme even when this principle is still present in a state of proserozyme. Consequently, finished thrombin acts as though it could bring about a remarkably quick conversion of proserozyme into serozyme, the process preliminary to the genesis of fresh thrombin being thus greatly hastened. The consequence is that, when thrombin is added to oxalated plasma which has been just recalcified, the total amount of thrombin this quantity of plasma is apt to furnish appears much more rapidly than it does when the same plasma is allowed to clot spontaneously without the stimulus of thrombin. In fact, thrombin itself thus accelerates the formation of thrombin. Owing to lack of time, I cannot report here in detail the experiments which have established this idea, but shall now consider briefly some of the views held by certain authors and which are not in agreement with the ideas developed above.

As is well known, my countryman, the physiologist Nolf, has adopted the rather startling theory of Wooldridge according to which, instead of being the immediate determining factor of coagulation, thrombin is generated as a consequence of the coagulation itself. According to Nolf, the transformation of fibrinogen into fibrin is not the effect, but the necessary condition of the appearance of thrombin. Many of the data which I have recorded above energetically plead against such a conception. For example, I would only recall the experiments showing the production of thrombin in fluids altogether devoid of fibrinogen, and thus proving unquestionably that fibrinogen does not play any rôle in the production of the coagulating principle.

One important point has been and still is controverted: I mean the true significance of the lipoid to which we have so often alluded. Schmidt, who had already observed the accelerating influence on coagulation, exerted by alcoholic extracts of tissues, believed that such lipoids rendered easier the production of thrombin, without assuming, as I do, that they really enter into its constitution. One of the most distinguished among the writers who have devoted their skill to the study of coagulation, Professor Howell, especially directed his attention towards the fact that the lipoid extracted, for example, from nervous tissue, is capable of inducing the coagulation of peptone plasma and hirudin plasma which, as is well known, remain fluid because they contain an anticoagulating substance, called antithrombin. In opposition to our assumption, Howell thinks that the lipoid is not a constituent of thrombin, but acts by neutralizing the antithrombin, which hindered the spontaneous conversion of prothrombin into thrombin. The real existence of antithrombin is of course unquestionable and it has been proved beyond doubt that antithrombin may be neutralized by thrombin, the two substances being, in all probability, capable of forming a compound. Now the question arises whether, when lipoid is added to peptone or hirudin plasma, the removal of the antithrombin function is due, as Howell claims, to the direct neutralization of antithrombin by this lipoid, or to a neutralization of antithrombin by thrombin generated under the influence of the same lipoid, the latter reacting with the serozyme or proserozyme also contained in the aforesaid plasma. In other words, according to this second interpretation, the neutralization of antithrombin by the lipoid would be merely apparent or at least indirect, the direct agent of this neutralization being the thrombin which the lipoid has caused to appear. I believe that such a conclusion is forced upon us by some recent and careful experiments by Gratia. Without entering into the somewhat complicated details, they have shown that the lipoid does not at all neutralize the antithrombin when the serozyme or proserozyme has been previously removed, that is, when the production of thrombin has been made impossible. Even when the lipoid is added in large excess, the abolition of the antithrombin function occurs only in proportion to the amount of serozyme present, that is, in proportion merely to the quantity of thrombin that can be generated. Consequently, a direct influence of the lipoid on the antithrombin cannot be admitted.

Furthermore, Howell's view could hardly be brought into harmony with a very essential fact, which has been mentioned above. Were his assumption correct, it should be admitted that serum yielded by the coagulation of recalcified oxalated plasma deprived of its platelets contains a large amount of antithrombin, since the addition of lipoid to such a serum, itself poor in thrombin, produces in this fluid plenty of the latter principle: upon the whole, the serum should in this respect resemble very closely the plasma from which it is derived. But, such being the case, it would be very difficult to understand why the lipoid neutralizes the antithrombin very quickly when added to serum, and very slowly when added to plasma. I think the only possible explanation of such a difference is that in serum but not in plasma, as was said before, the serozyme is capable of reacting very rapidly with cytozyme to generate thrombin. However, the question as to the relation of cytozyme to the antagonistic function is one of the most delicate in the whole study of coagulation and I fully realize that different views may still be upheld. As I told you at the beginning, coagulation has been studied for years and years by many investigators; none of them can presume that the problem is yet solved; every one of them merely indulges in the hope of gathering some complementary data; a little more information.

JOHNS HOPKINS HOSPITAL BULLETIN

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ON CERTAIN VARIATIONS IN THE FORM OF THE HUMAN ELECTROCARDIOGRAM

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Einthoven[5] pointed out that the form of the human electrocardiogram varies with respiration and with the position of the body. Waller[7,8] independently made many observations to the same effect. These variations consist for the most part of changes in the amplitude and sometimes of the direction of the waves of the QRS complex. They have been shown to be related to changes in the electrical axis of the heart in accordance with developments growing out of Einthoven's law that lead II is equal to the sum of leads I and III. The subject from this point of view is summarized in a recent article[3] from this laboratory. The purpose of this communication is to point out more clearly certain peculiarities of these and other variations in the form of the electrocardiogram which have a practical bearing.

The variations which we are to discuss are seen very frequently in lead III and this fact has led to a feeling that the findings in this lead are unimportant, or may even be wholly ignored. Such a policy is not warranted by the explanation of the changes which may occur, and tends to prevent us from gaining the most that can be learned from the interpretation of the electrocardiographic records.

The commonest variation in the electrocardiogram is that the amplitude of R in lead I increases with expiration and decreases with inspiration, whereas R in lead III behaves in the opposite manner (the fact that an S in lead III acts as an algebraically negative R should be kept in mind). As stated above, this conspicuous variation is commonly most marked in lead III and it is often possible, as is well known,[9] to change a positive to a negative wave in this lead by deep respiration or by a change in position of the subject. An example of this reversal of lead III is given in Fig. 1, in which the change is due to a shift of position. In this instance the value of lead III when the patient was on his right side (a) is -5 mm., and when he turned on his left side (b) it is $+5$ mm. (measured at corresponding points of the respiratory cycle).

In examining routine records it was noticed that the same changes are not infrequently present in leads I and II in different cases. So far as is known this has not been commented upon. Fig. 2 is an illustration of reversal of direction in lead I. When the subject was prone (a) the value of lead I (in expiration) is $+5$ mm., and it is -3.5 mm. when the left lateral decubitus is assumed (b). In addition the wave becomes deeply notched. An instance of marked change in lead II appears in Fig. 3. In lead II during one phase the value of R is 13 mm., and during another phase it is 9 mm., while at the same time a marked notching appears. *Variation in amplitude, as might be expected, is much more frequent than reversal of direction; it is also seen not only in lead III but in the other leads in appropriate cases.*

Of the same nature as these peculiarities of the electrocardiogram, at least from our present point of view, is that lead in which there is practically no deflection of the galvanometer string from the iso-electric line during the time corresponding to the QRS interval. This phenomenon is seen not infrequently in one of the three leads, and there are many examples in leads I and II as well as in lead III. Fig. 4 illustrates the phenomenon in each of the three leads (the records are from different individuals). It happened that these records were all taken within a period of five days. It may be remarked that in the presence of this peculiarity the two leads which are not iso-electric, or of very low potential difference, have approximately equal values. It is of importance that the occurrence of these practically zero values, when confined to a single lead, is not in itself of any significance, prognostic or otherwise, as will be readily appreciated from the explanation of the phenomenon given below.

Notching of R or S depends upon the manner in which the electrical axis shifts, as was shown by Williams.[10] Such notching is sometimes transient (see Figs. 2 and 3), and examples of this peculiarity in each lead are fairly numerous. Since the same principle must underlie the phenomenon whether it be transient or persistent, the significance to be attached to notching of a given degree is not decreased by transiency. It also follows that *the emphasis which is to be put on the notching does not depend upon the lead in which it presents itself.* When the principles of the electrical axis are borne in mind, a careful examination of the three leads practically always shows signs in all leads of the factors underlying the notching. Fig. 5 shows progressive change in the notching of leads I and III which is merely a reflection of shifts of the electrical axis. Evidence of the notching is present all through the records and analysis of the shift of the axes shows that the fundamental course of the axis remains the same. The stability of lead II is noteworthy.

When patients are followed by taking electrocardiographic records in the standard method at intervals, similar changes are often observed in one or the other lead.

The mechanism of these changes, as far as it is known, has to do with the position of the electrical axis of the heart during its rotation. When the electrical axis is nearly perpendicular to the plane of a given lead, slight changes in the angle of the axis give rise to relatively wide variations in the value of that lead. For example, a shift of 5° from 80° to 85° halves the value of lead I, while a shift of the same extent from 5° to 10° decreases the amplitude of lead I by only 1.1 per cent (see 3, table 2). The striking changes of reversal of negativity

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illustrated above occur when the electrical axis is very nearly perpendicular to the plane of the lead affected and is carried over to the other side of the perpendicular by the shift which occurs. The appearance and disappearance of notching of R or S is to be explained on the same basis.

In a given case which shows variation in amplitude, reversal in direction of R or S, or transient notching, the peculiarity is marked in one lead and present to a less extent in another, while it is practically absent from the remaining lead. This follows from the relation which exists between the planes of the leads as shown by Einthoven,[4] namely, that of the sides of an equilateral triangle. It is convenient to refer to the lead or leads which show relatively marked changes as *unstable*, while those which do not vary may be said to be *stable*.

The original exposition of this subject by Einthoven[6] sets forth that the leads change their values from positive to negative and vice versa at the following angles of the electrical axis to the horizontal plane: lead I at $+90°$ * and $-90°$; lead II at $+150°$ and $-30°$; and lead III at $+30°$ and $-150°$. When the electrical axis is very close to one of these angles and undergoes but little shifting the corresponding lead is of approximately zero value. Table 1 gives for each

TABLE 1.—SHOWING THE ANGLES BETWEEN WHICH A SHIFT OF 5° CHANGES THE LEAD VALUE 18% OR MORE

Leads	Angles	
I	+ 65° to +115°	—115° to — 65°
II	+125° to +175°	— 55° to — 5°
III	+ 5° to + 55°	—175° to —125°

TABLE 2.—SHOWING THE ANGLES BETWEEN WHICH A SHIFT OF 5° CHANGES THE VALUES OF TWO LEADS AT LEAST 10%

Leads	I+	II−	III+	III—
I+	....	....	+50° to +70°	....
I—	....	+110° to +130°	....	—130° to —110°
II—	—70° to —50°	....	+170° to —170°	....
III—	....	—10° to +10°	....	....

of the leads the angles between which a shift of the electrical axis to the extent of 5° changes the value of the lead 18 per cent or more. The angles between which a similar rotation of 5° affects the values of two leads at once to the extent of at least 10 per cent are shown in Table 2. These tables are arranged on the basis of the table for the calculation of the "manifest value" recently published from this laboratory (3, Table 2). They are offered with the hope that they may help make useful a knowledge of the peculiarities of the electrocardiogram under discussion.

* The custom of designating the angle "*a*" as positive when below and negative when above the horizontal plane is followed. For methods of determining this angle consult (2) and (3).

Examination of these tables shows why variation of the QRS group is more commonly seen in lead III. It is because normal hearts as a rule have electrical axes which lie between $+30°$ and $+70°$ (although the normal limits are somewhat wider), limits within which lead III varies conspicuously. On the other hand, in cases of ventricular preponderance in which the angle of the electrical axis is negative or greater than $+90°$ it is chiefly lead II which is unstable (see tables). It was essentially for this reason that the suggestion was made[1] to use leads I and III for the determination of the angle of the electrical axis in cases of suspected preponderance.

The electrocardiographic variations described may be divided into two groups, namely, those which occur in a very brief period, often during the inscribing of the record; and those which are first found after the lapse of a relatively long interval.

With regard to the underlying cause of the variations our knowledge is very imperfect. Simple variation in amplitude and change in direction and appearance and disappearance of notching during the taking of the record are no doubt due for the most part to the effect of respiration on the heart, as was long ago pointed out. These changes can also often be brought about in unstable leads by altering the position of the body from the prone to the left or right lateral position. In a few cases such conditions as gastric distention, ascites, hydrothorax and pneumothorax may play a part. Doubtless it is in a sense accidental that the electrical axis in a given instance happens to fall so close to the proper angle as to cause almost no deflection in the corresponding lead.

Marked variations of the nature described, beyond the possibility of explanation on the basis of a change in the relative position of the heart, often occur especially with lapse of a longer interval. These changes are of course, of much greater significance. Their full explanation is wanting as yet. In addition to the occurrence of definite pathological changes in the conduction system, disturbances in the relation between the ventricles as to mass and perhaps as to volume, are thought to play a prominent part. It is quite possible that local metabolic changes may be a factor. It is not to be concluded that the facts presented are supposed to prove a constant relation between the electrical and the anatomical axis of the heart although there is no doubt a definite relation of some nature between them.

It is further of importance to keep in mind the fact that the calculated electrical axis represents essentially the resultant of the constantly shifting axes during the spread of the excitation wave, and does not give us any clue as to the actual degree or direction of the rotation that may have taken place during the inscription of the QRS complex. This can be determined only by exact analysis from given time instants with all three leads accurately in phase.

Emphasis should be laid on the occurrence of these peculiarities in all the leads, and particularly on the fact that they are very often present in lead II in pathological hearts.

The principles applied in this paper to the consideration of the QRS complex in the three leads are also applicable to

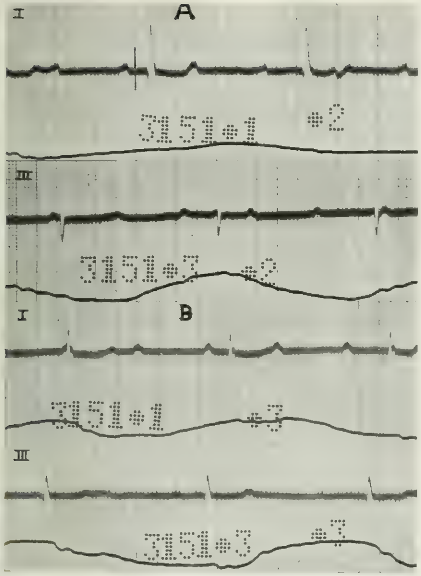

FIG. 1.—Showing alteration in amplitude of lead I and reversal of direction with change of position. (A) Subject lying on the right side; (B) lying on the left side.

FIG. 2.—Showing reversal of direction of lead I and variation in the notching of leads I and II with change of position. Note also the variation in amplitude. (A) subject lying on his back; (B) lying on the left side.

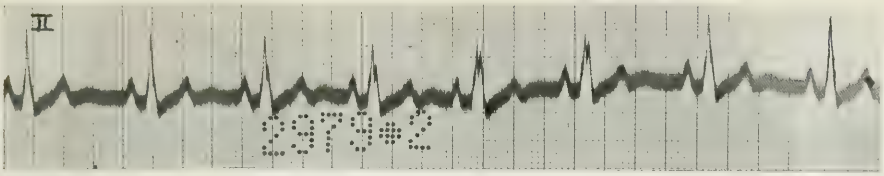

FIG. 3. -Showing variation in amplitude and notching of lead II.

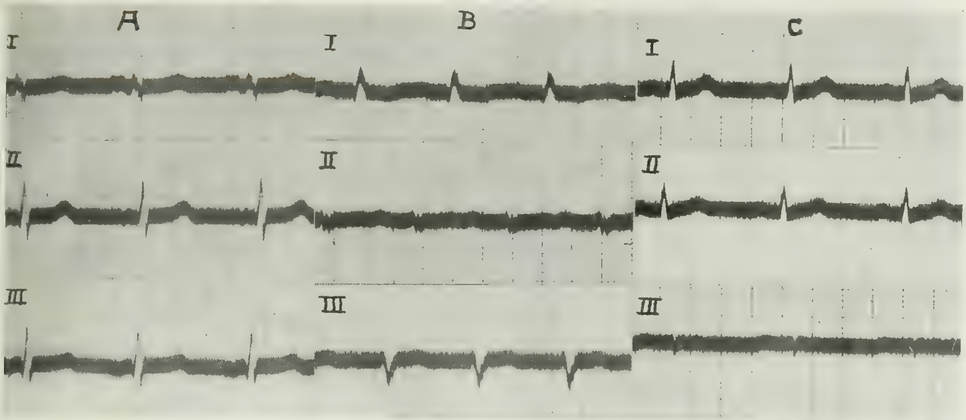

FIG. 4.—Showing leads of approximately zero value: (A) Lead I; (B) lead II, (C) lead III.

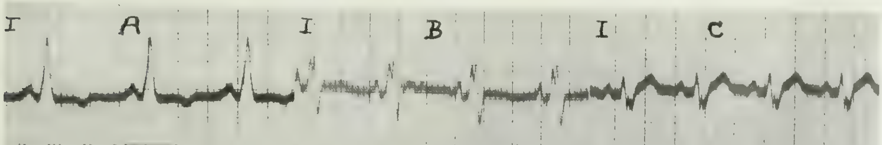

FIG. 5. -Showing progressive change in the notching of leads I and III. Note the stability of lead II. (A) October 28, 1920; (B) February 1, 1921; (C) March 21, 1921.

similar changes undergone by the "P" and "T" waves of the human electrocardiogram. Studies dealing with these latter waves in this connection are under way at the present time in this laboratory.

SUMMARY

1. Variations in amplitude (including extremely low values) and direction of the QRS complex and transient notching of R and S in the human electrocardiogram occur not only in lead III but also not uncommonly in the other leads, especially in records of abnormal hearts.

2. These peculiarities are dependent upon the relation of the electrical axis of the heart to the planes of the leads. Tables are given showing the angles at which conspicuous variation is present in each lead, and the angles between which fairly marked variation occurs in each combination of two leads. The terms "stable" and "unstable" are suggested with reference to the variability of the leads.

3. Variations are due in some instances to shifts in the relative position of the heart; in others to pathological changes in the bundle tract and to alteration in the relation between the ventricles; in a great many others to causes at present unknown.

4. No significance is to be attached to curves of low amplitude in any one lead only.

5. In the light of the evidence set forth above, it is not justifiable to assume that the findings of lead III can be disregarded.

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A STATISTICAL NOTE ON EPIDEMIC ENCEPHALITIS*

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During the last years of the war and since there has occurred the significant beginning and menacingly rapid spread of a disease either previously not existing, or at least not occurring frequently enough to get general recognition, namely, *encephalitis lethargica* or epidemic encephalitis. Because of its extremely rapid development in frequency of occurrence and because of its extremely fatal character, together with the probability that it may become an epidemic disease of an importance similar to that of poliomyelitis it seems desirable, thus early in its history, to make some analysis of its statistical characteristics. To do this has been the object of the preparation of this paper, in which I have been assisted on the arithmetic side by Mr. John Rice Miner.

The aid which the mathematical analysis of statistical data can render the epidemiologist and pathologist, and the degree of certainty which such analysis can add to their conclusions, are not generally recognized. Usually the health officer is content with the mere tabulation of frequency of occurrence of morbidity and mortality. Such figures are too frequently made the basis of conclusions without any recognition of the errors of sampling involved. Before one can be in any degree certain of conclusions from such figures their probable errors must be known.

Material.—Two papers have recently appeared giving raw data regarding the development of epidemic encephalitis. The first of these papers[1] deals with the cases of the disease in the whole United States in the years 1918 and 1919. The second paper[2] deals with the much more restricted field of New York City in 1919 and 1920, but because of the accelerated frequency of occurrence of the disease during the latter year, contains far more statistical material than Smith's paper. It is with the data from this second paper that we shall chiefly deal here.

Case Fatality Rate.—Table 1, taken from (2), gives the incidence and deaths from this disease in New York City in 1919 and 1920. There is a slight correction of an obvious arithmetic error in the total deaths in 1919, from that given in the original.

These data give the following case fatality rates:

$$1919, \text{ case fatality rate} = \frac{33 \times 100}{128} = 26 \text{ per cent}$$

$$1920, \text{ case fatality rate} = \frac{211 \times 100}{565} = 37 \text{ per cent}$$

The first of these figures agrees well enough with the 29 per cent given by Smith for the case fatality rate over the whole country in the years 1918 and 1919. The significant thing is that the case fatality rate appears to be increasing. Such a conclusion depends upon the assumption that morbidity from this cause was at least as well reported in 1920 as in

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1919. It may be accepted with reasonable certainty that the *deaths* were as well reported in the one year as the other. The well-known high standards maintained in the work of the Bureau of Vital Statistics of the New York City Department of Health practically ensures this. The morbidity figures are more doubtful, because this disease has only recently been made reportable in the city. But let us carefully examine the alternatives which are presented. If we assume (a) that the deaths have been equally well reported in 1919 and 1920, which is a justifiable assumption, and (b) that the true, but unknown,

TABLE 1.—INCIDENCE AND DEATHS FROM EPIDEMIC ENCEPHALITIS, BY MONTHS, 1919-1920

Month	1919		1920	
	Cases	Deaths	Cases	Deaths
January	5	..	36	12
February	16	..	149	50
March	25	9	116	52
April	10	1	66	16
May	16	5	42	22
June	4	1	20	12
July	2	1	39	9
August	1	..	28	11
September	8	5	22	6
October	23	7	11	3
November	9	3	12	9
December	9	1	24	9
Totals	128	33	565	211

case-fatality rate has remained absolutely constant at the 1919 figure during these two years, then it follows that to get the 37 per cent observed, it will be necessary to suppose that the 565 cases reported in 1920 far *underestimate* the true number which occurred. It must be assumed that actually 812 cases occurred instead of the 565 reported. Now it would seem altogether unlikely, indeed indefinitely improbable, that in the year 1920 there occurred in New York City 247 cases of encephalitis lethargica about which the Department of Health did not know. The *prima facie* reason why this is so improbable is the ever-increasing interest and recognition of the importance of this disease by the Department of Health during the period named.

Suppose we consider the second alternative which is that the figures for 1919 greatly underestimate the true and assumed constant case fatality rate. This means, if we assume the deaths to have been substantially accurately reported for the same reason as before, that there were more *cases* reported in 1919 than actually occurred. To justify this second alternative it must be supposed that actually there occurred in 1919 in New York City only 89 cases of epidemic encephalitis, instead of the 128 reported. This would be a highly improbable assumption. And further we must reckon with the close agreement between Smith's figures of 29 per cent for the case fatality rate for the whole country and the New York 1919 figures of 26 per cent.

Altogether it may be tentatively concluded, with a high degree of probability, that the case fatality rate from the disease is on the increase. But the New York numbers are absolutely small in a statistical sense, and the further question must be asked, as to whether the 11 per cent increase in the case fatality rate between 1919 and 1920 in New York is statistically significant, *i. e.,* greater than might reasonably be supposed to have arisen from chance fluctuation alone.

The problem may be stated in this way: If in a sample of 128 cases of the disease 33 deaths occur, what would be the number of deaths expected in a sample of 565 cases, if nothing but chance were involved in the matter, or in other words if there had been no change in the true case-fatality rate?

We have, by well understood principles (cf. 4 and 5):

Mean deaths expected in second sample of $565 = 148 \pm 16$.

Or, in other words, it is an even chance, if chance alone caused in 1920 fluctuations from the case-fatality rate observed in 1919, that from 132 to 164 deaths would have occurred in 1920, rather than a number smaller than 132 or larger than 164. Actually 211 deaths occurred in 1920. The difference between 211 and 148 is more than 3.9 times the probable error of the latter figure. Whence we may conclude that there was a statistically significant rise in the case fatality rate in 1920 as compared with 1919 in New York City.

Seasonal Distribution.—A question which at once occurs to one in examining Table 1 is this: Is the seasonal distribution (by months) of cases changing as the disease grows in importance, or is the monthly distribution in 1920 different from that of 1919 only by as much as might reasonably be expected from chance sampling?

To determine this question it is necessary only to determine the probability that the two distributions differ significantly from each other. It has been shown by Pearson [5, 6, 7] that such a probability will be given by the expression

$$P = \frac{\int_{\chi}^{\infty} \chi^{q-2} e^{-\frac{1}{2}\chi^2} d\chi}{\int_{0}^{+\infty} \chi^{q-2} e^{-\frac{1}{2}\chi^2} d\chi}$$

Where

$$\chi^2 = NN'S\left[\frac{(f/N - f'/N')^2}{f+f'}\right]$$

the summation S extending over all pairs of the elemental frequencies f and f'.

From the data of Table 1, we get for the probability that the 1920 and 1919 distribution are two random samples from the same unchanged phenomenal universe

$$\chi^2 = 89.542,$$

whence it is easily determined that

$$P < .000,000,1.$$

Or, in words, it appears that less often than once in 10 million trials would one expect to get true samples of the size here dealt with, as divergent as are the 1919 and 1920 monthly distributions, from the operation of chance alone. Hence we conclude that there was a significant difference in the seasonal incidence of the disease, as indicated by the monthly distribution of cases, in 1919 as compared with 1920 in New York. Examining the details of the χ^2 calculations it is seen that the significant divergences are in the autumn and the spring. There was a significant flare-up in the spring of 1920 lacking in 1919, and a significant autumn outbreak in 1919 lacking in 1920.

Time Lag of Deaths.—On the basis of the 24 months' experience exhibited in Table 1 it is possible to determine the average time that deaths lag behind cases. Put in another way this means the mean duration of *fatal cases* of encephalitis lethargica, between recognized onset and death. This is found to be:

Mean duration of time
between onset and death $= 1.046 \pm .230$ months.

The epidemiological significance of this datum is that it may be expected, in so far as the 1919 and 1920 experience of New York City may be regarded as typical, that the peak of mortality in an epidemic outbreak of this disease will probably occur from 23 to 37 days after the peak of morbidity has been passed. The contrast of this with the time lag in epidemic influenza is sufficiently striking.

Influence of Sex.—Table 2, again taken from (2), gives the sex and age distribution of the cases and deaths in New York City in 1920.

From these data the first question to which attention may be directed is this: Is the difference between the sexes in respect of (a) incidence, or (b) fatality, greater than might reasonably be expected to arise from chance alone, in a city having the sex distribution of its population that New York has?

By elementary principles of probability it is easily found that, among those persons having the disease in New York in 1920, the probability that a given person picked at random would be a male is given by

$$p = \frac{316}{549} = .5756.$$

whence

$$\frac{pq}{n} = .000445.$$

In the whole population of New York (on the basis of the 1910 census figures) the probability that any person picked at random will be a male is

$$p' = .4994 \text{ and}$$
$$\frac{p'q'}{n'} = .000,000,001$$

whence

$$p - p' = .0762 \pm .0142$$

and

$$\text{Diff./P.E. Diff.} = 5.4.$$

Or, it appears that there is a significantly larger proportion of males among those attacked by epidemic encephalitis than there is among the general poulation. A deviation as large as that between p and p' would occur on the basis of chance alone only once in about every 10,000 trials.

TABLE 2.—ENCEPHALITIS CASES AND DEATHS, BY AGE AND SEX, 1920

Ages	Cases			Deaths		
	Male	Female	Total	Male	Female	Total
Under 5 years	23	24	47	10	8	18
5– 9 years.......	26	14	40	10	2	12
10–14 "	32	18	50	6	5	11
15–19 "	23	24	47	5	5	10
20–24 "	37	34	71	13	11	24
25–29 "	29	27	56	11	14	25
30–34 "	35	18	53	8	8	16
35–39 "	30	18	48	14	11	25
40–44 "	19	14	33	7	10	17
45–49 "	24	16	40	13	7	20
50–54 "	15	8	23	8	3	11
55–59 "	8	3	11	6	1	7
60–64 "	7	7	14	3	5	8
65–69 "	4	4	8	2	1	3
70–74 "	3	2	5	2	1	3
75–79 "	0	1	1	0	1	1
80–84 "	1	1	2	0	0	0
85 years and over.	..	..	..	..	..	..
Totals..........	316	233	549	118	93	211

Among those *dying* from epidemic encephalitis in New York City in 1920 the probability that any individual picked at random would be a male was

$$p = \frac{118}{211} = .5592.$$

Whence

$$\frac{pq}{n} = .001168$$
$$p' = .4994 \text{ as before.}$$
$$p - p' = .0598 \pm .0230.$$

From this result we may conclude that the proportion of males dying from epidemic encephalitis in New York in 1920 was not significantly different from that found in the general population.

It thus appears that more males were attacked but no more died from encephalitis than would have happened if sex had no differential effect whatever relative to this disease. This

result can only mean that the case fatality rate is different in the two sexes.

Influence of Age.—It is stated in (2) that "No age seems exempt, but the greatest proportion of cases occurred in young adults, with a preponderance of males." This raises the following question: Is the age distribution of each of (a) male cases, (b) female cases, (c) male deaths, and (d) female deaths, significantly different, as a whole, from (e) the male population age distribution and (f) the female population age distribution?

Using the χ^2 method, and taking the 1910 age distribution of the New York City population as the basis of comparison, one gets:

For male *cases*:

$$\chi^2 = 8.254.$$
$$P = .410.$$

Or, one would expect in 4 out of every 10 trials with samples of the size here dealt with to get a divergence as great as or greater than that between the male case incidence age distribution and the general population age distribution, if epidemic encephalitis were perfectly random in its age incidence. In other words, there is slight basis for assuming that this disease attacks males more frequently at one age than another.

If we leave out the "under 5" group

$$\chi^2 = 4.111.$$
$$P = .767.$$

This means that in males 5 years old and over the age incidence of cases is very close indeed to a random one. In 77 out of every 100 trials there would result from chance alone deviations as great as or greater than that actually shown between male cases and the general male population.

Turning to male *deaths* we have:

$$\chi^2 = 18.450.$$
$$P = .018.$$

These values indicate a significant divergence of the male death age distribution from the general population male age distribution. But a further analysis gives:

For male deaths under 45 years of age:

$$\chi^2 = 5.790.$$
$$P = .448.$$

For male deaths under 35 years of age:

$$\chi^2 = 3.319.$$
$$P = .652.$$

It thus appears that it is chiefly the excess of male deaths beyond age 45 from this disease that causes the low value of P for the entire male death distribution.

Putting all the facts together it can be asserted, on the basis of the experience of New York City in 1920 with epidemic encephalitis, that in males the disease is probably not significantly more likely to attack one age than another, but instead falls upon males in about the proportion that they are normally present in the general population. It is furthermore not more fatal among the attacked at one age than another until after age 35 is passed. From that point on there are more fatal cases than would be expected if the toll of death were taken purely at random in respect of age.

Turning to the age distribution of female *cases*, and again comparing with the age distribution of the total female population (as of 1910) we get:

$$\chi^2 = 4.847.$$
$$P = .773.$$

It is thus quite certain that, so far as the experience of New York City in 1920 may be taken as typical, epidemic encephalitis occurs among females without definite age preference, striking the different ages in due proportion as they are represented in the general population. Females of any particular age are not more likely to contract the disease than females of any other age.

The age distribution of *deaths* among females presents a different case. It will be well to examine the facts in some detail. Table 3 gives the data.

TABLE 3.—DATA FOR TESTING THE DEVIATION OF THE FEMALE DEATH AGE DISTRIBUTION FROM THE FEMALE POPULATION AGE DISTRIBUTION

Ages	m Age distribution of female population (as of 1910)	m' Age distribution of females dying of encephalitis lethargica in 1920	$\frac{(m-m')^2}{m}$
Under 5 years......	9.810	8	.3340
5– 9 years........	8.553	2	5.0207
10–14 "	8.268	5	1.2917
15–19 "	9.427	5	2.0790
20–24 "	10.954	11	.0002
25–34 "	17.495	22	1.1600
35–44 "	13.044	21	4.8526
45–64 "	12.554	16	.9459
65 years and over..	2.894	3	.0039
Totals...........	92.999	93	15.6880

$$\chi^2 = 15.6880$$
$$P = .048.$$

It thus appears that the death distribution as a whole is significantly divergent from what would happen if the disease was as likely to be fatal at any given age as at any other. But it is also apparent that more than half of the large value of χ^2 is due to the contribution of but two age groups. These age groups are the 5 – 9 and the 35 – 44. The deaths in the former were fewer than expected and more than expected in the latter. The numbers are small, however, and it will probably be found, when more data are available, that the facts as to age distribution of deaths are the same in females as in males, namely that there is a definite tendency towards greater fatality in the higher age groups.

Summary

It is distinctly to be understood that the results herein set forth have only such degree of validity as inheres in the experience of New York City with epidemic encephalitis during the two calendar years 1919 and 1920. Doubtless as more material accumulates new statistical relations of this disease will appear. But at the present moment the data here discussed are the most extensive in existence, so far as the writer knows. They fairly represent, in short, about all the basis of a statistical character which exists for forming an intelligent opinion *now* about the statistical features of this potentially important disease. The conclusions reached are, of course, subject to modification by more extensive experience.

What these conclusions are may be summarily stated as follows:

1. In the year 1920 the case incidence of epidemic encephalitis increased in New York City nearly five-fold over 1919. At the same time the case fatality rate increased from 26 per cent of the attacked to 37 per cent of the attacked. It is believed that this increase in case fatality rate is a real phenomenon, and it is certainly statistically significant.

2. The seasonal incidence, as judged by monthly distribution of cases was significantly different in 1920 to what it was in 1919.

3. The peak of mortality in an epidemic outbreak may be expected to occur from 23 to 37 days *after* the peak of case incidence (morbidity).

4. There is a significantly larger proportion of males among the attacked than there is in the general population, or, put in another way, males are especially susceptible, to a statistically significant degree.

5. Deaths occur among males no more frequently in comparison with females than would be expected from the normal proportions of the two sexes in the population at large.

6. The disease is not more likely to attack either males or females at one age than at another. The age distribution of attacked cases, in other words, does not significantly differ in either sex from the age distribution of the general population.

7. The age distribution of deaths does differ significantly in both sexes from the age distribution of the population. There appears to be a definite tendency for the disease to be more fatal in the higher age groups.

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THE THERAPEUTIC SIGNIFICANCE OF THE GRAM REACTION

By John W. Churchman

Attention was called in 1912,[1] and in a number of publications which appeared in the following years, to a striking parallelism which exists between the gentian violet reaction and the Gram reaction. If organisms whose growth is inhibited by gentian violet be spoken of as gentian positive, and those whose growth is uninfluenced by the dye as gentian negative (Figs. 1 and 2), it is found that the large majority of Gram-positive organisms are gentian positive, while the large majority of Gram-negative organisms are gentian negative. The actual figures are as follows:

		Growth inhibited	Growth uninfluenced
130 species	Gram-positive 77	70 (90%)	7 (9%)
	Gram-negative 53	8 (15%)	45 (84.9%)
318 strains	Gram-positive 182	165 (95%)	17 (9.3%)
	Gram-negative 136	15 (11%)	121 (88.9%)

[1] Jour. of Exp. Med., 1912, XVI, No. 2, p. 221.

Moreover, it is just the organisms which are constantly and definitely Gram-positive (like *B. subtilis*) which are constant in their failure to grow even in the presence of minute quantities of the dye; and just the organisms which are constantly Gram-negative (like *B. coli*) which grow readily, even in fairly strong dilutions of the dye. (See Figs. 1 and 2.)

Rather striking, too, is the difference in avidity of the living Gram-positive and Gram-negative organisms for the stain, when examined in the hanging drop: *B. subtilis* rapidly becomes deep violet and promptly loses its motility, while *B. coli* stains slowly—some of the individuals very slowly—only a small proportion of the individuals stain at all deeply, and all retain their motility for some time. The criticism that (since the Gram reaction is a phenomenon exhibited by dead bacteria, while the gentian reaction is one exhibited by living bacteria) to stress the parallelism between the two reactions is unduly to magnify properties exhibited by living matter, on the one hand, and dead matter, on the other, was met by per-

forming the Gram stain on living organisms centrifugated in the test tube: The parallelism was again found to hold.

Facts of this sort made it natural to seek an explanation for the parallelism between Gram and gentian reactions in the assumption that both depended on the presence in the Gram-positive organisms, and the absence from the Gram-negative organisms, of chemical groups avid for certain chemical groups in the molecule of the stain. Saturation of these groups (Ehrlich's "fixation") would, according to this theory, lead to retention of the dye by the Gram-positives and their death when exposed to it; absence of these groups from the Gram-negatives would account for their incapacity to retain the dye by Gram's method and their ability to grow in its presence. This supposition received added support from the further observation that the selective bacteriostatic power was possessed not only by gentian violet but also by other members of the tri-phenyl-methane group: both *the dyes* belonging to this series, and *simpler substances* with similar molecular structure (Fig. 5). Evidence has, however, gradually accumulated which proves this hypothesis to be incorrect.

(1) In the first place, the parallelism between Gram reaction and gentian reaction—while striking—is not complete. There is a small but definite number of species in each group which do not follow the rule (Figs. 3 and 4).

(2) More important than this is the observation of a Gram-negative strain occurring in nature, which, although all other strains of the same species followed the rule and grew well in the presence of gentian violet, would not grow when the dye was present in the media on which it was planted.[2] This strain of *B. enteritidis* is shown in Fig. 6, stroked on a divided gentian violet plate along with the other four strains of the same species studied. Its exceptional behavior will be clear at a glance. Furthermore, it was not transient. It was observed in the two samples of this strain examined. Both samples were descendants of a strain isolated in Cambridge ten years before, and had been kept growing by hundreds of transplants in two different laboratories. Yet both had retained this remarkable inherent susceptibility to gentian violet which by no possibility could have been acquired by struggle against the dye. This strain of *B. enteritidis* was identical, by all morphological, tinctorial and cultural tests, with the other strains studied; it differed from them only in its growth-behavior toward gentian violet; it was as definitely Gram-negative as the other strains. This strain may be referred to as a "Strain-within-a-species" variant.

(3) It has recently been possible to isolate from a "pure" culture of *B. coli* a "strain-within-a-strain" variant which will not grow in the presence of gentian violet.[3] I have repeatedly made the statement that the growth of a Gram-negative organism, such as *B. coli*, proceeds on the gentian side of a divided plate as luxuriantly as it does on the plain agar. Such a statement would certainly seem to be warranted by an experiment like the one represented in Fig. 2, where *B. coli* has run wild over the gentian violet agar (as though it actually found the dye a growth accelerator), while *B. subtilis*, as usual, shrinks from it.

Further investigation of this subject, however, by quantitative methods, demonstrates that, if the experimental conditions be altered as regards the number of bacteria transplanted, the statements as to the effect of the dye on Gram-negative organisms must be considerably modified. If a divided plate be stroked with a 24-hour broth culture or a heavy suspension of *B. coli*, growth is equally luxuriant on the two sides of the plate (Fig. 7A). If, however, increasingly weak suspensions of the organism be used for the stroking, fewer and fewer colonies appear on the gentian violet agar and at high dilutions of bacterial suspension none at all may appear (Fig. 7, B and C). By separate cultivation of the colonies which appear on the plain agar side of such plate it has been possible to isolate from a "pure" culture of *B. coli* a gentian-positive and a gentian-negative strain. These two strains, labelled for convenience strain X and strain Y, show their difference in behavior toward gentian violet, whether they are stained and then planted on plain agar or are stroked across divided gentian violet plates. This specific characteristic was not transient, for the experiment illustrated in Figs. 8 and 9 was done after the two strains had been allowed to grow for six weeks through a large number of transplants, and it will be seen that the original features of the two strains have been preserved. Cultural studies of these two strains showed them to be identical in every respect. These facts may be interpreted as follows:

There may exist within a single bacterial strain two types of individuals which, although in every other morphological, tinctorial and cultural characteristic identical, are quite dissimilar in their reaction toward gentian violet, one growing vigorously and the other not at all on media containing this dye. The latter we may refer to as a "strain-within-a-strain" variant.

No observations of variants, which at all approach these in nicety of selective behavior toward bacteriostatic agents, have hitherto been made in the bacterial field. One may speak of the phenomenon presented as reversed fastness, inasmuch as unlike the usual type of fastness seen in the protozoan field in which it is the ordinary run of organisms which are susceptible and the occasional organism which is fast, in this instance it is the ordinary run of organisms which are fast and the occasional organism which is susceptible.[4]

The variant strains of *B. enteritidis* and *B. coli* just referred to are definitely Gram-negative. *The factor which determines the reaction of an organism toward the Gram stain is not necessarily, therefore, the same as the factor which determines its cultural behavior toward gentian violet; and the chemical affinity hypothesis must be abandoned as the sole explanation of the parallelism between Gram and gentian reactions.*

[2] Jour. of Exp. Med., 1912, XVI, No. 6, p. 822.

[3] Jour. of Exp. Med., 1921, XXXIII, No. 5, p. 569.

[4] It is interesting to note that, after the report of the variant type of *B. enteritidis* and of the gentian-positive strain of *B. coli* just described, De Kruif has reported as an instance of microbic dissociation the isolation, from a single culture of *B. lepisepticum* of two strains, one of which was virulent and the other avirulent.

FIG. 1.—Divided gentian violet plate stroked with Gram-positive and Gram-negative organisms to show that the growth of the former is prevented by the dye and the growth of the latter uninfluenced. *C*, *B. coli; O, Oïdium albicans; A, M. aureus; T, B. typhosus.*

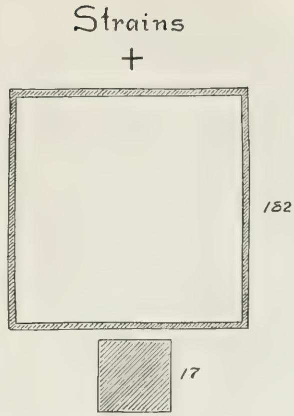


FIG. 3.—Schematic representation of effect of gentian violet on Gram-positive organisms; study of 182 strains. The central white area represents the number inhibited by the dye (165), the shaded border the number unaffected (17). The area of the shaded border is shown in the shaded square.

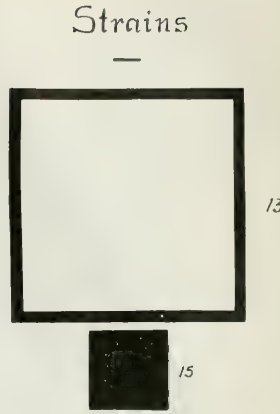


FIG. 4.—Schematic representation of effect of gentian violet on Gram-negative organisms; study of 136 strains. The central white area represents the number unaffected by the dye (121); the black rim the number killed (15). The area of the black border is shown in the black square. It will be noted that the facts are just the reverse of those for the Gram-positives.

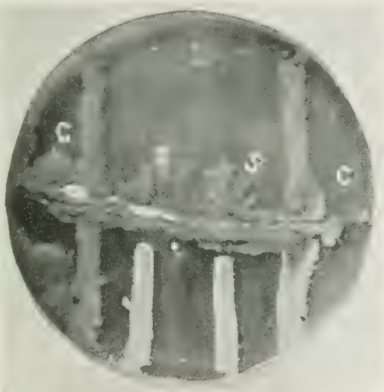


FIG. 2.—Note that the Gram-negative *B. coli* (*C*) has spread wildly over the gentian agar, while the Gram-positive *B. subtilis* (*S*) shrinks from it.

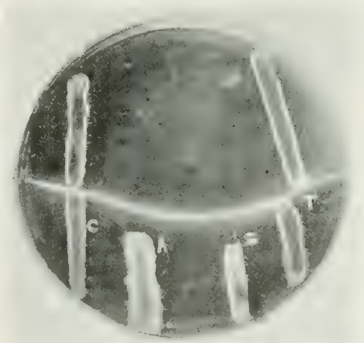


FIG. 5.—Experiment to show that a dye chemically related to gentian violet (Dahlia) possesses a similar selective bacteriostatic power. *C, B. coli; A, B. anthracis; S, M. aureus; T, B. typhosus.*

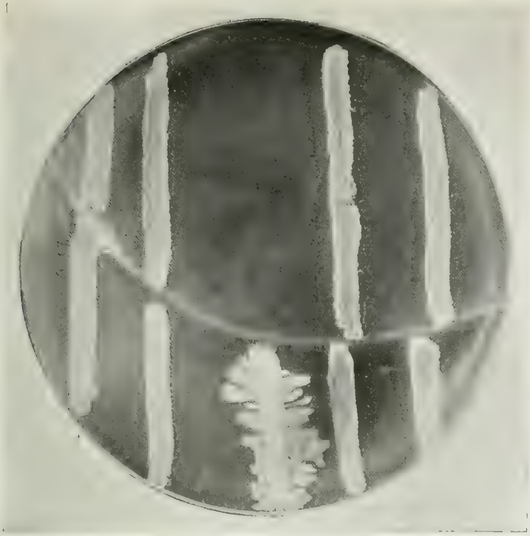


Fig. 6.—Divided gentian violet plate, showing a strain-within-a-species variant. Five strains of *B. enteritidis* have been planted; all except one have grown on the gentian violet agar.

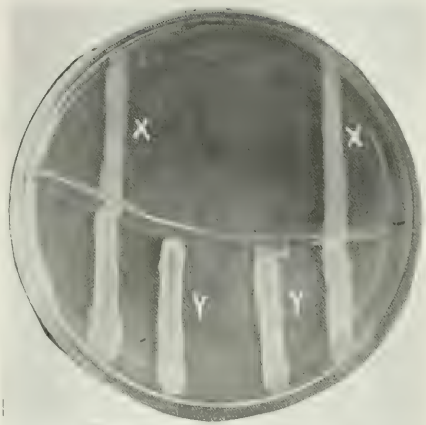


Fig. 8.—Divided gentian violet plate stroked with two strains isolated from a single "pure" culture of *B. coli.* The growth of strain *X* is uninfluenced by the dye; that of strain *Y* is entirely prevented. Strain *Y* is referred to as a strain-within-a-strain variant. The two strains are identical by all other tests.

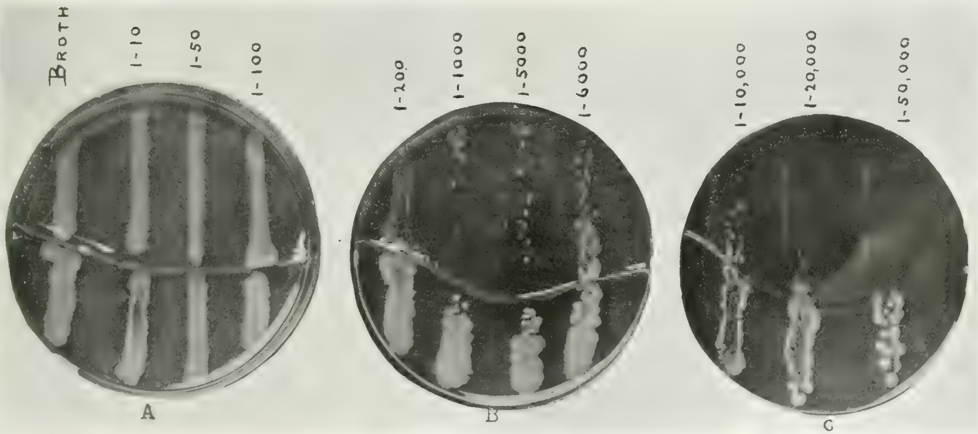


Fig. 7.—Divided gentian violet plates, stroked with increasingly weak bacterial suspensions. Note that in Plate A growth is equally good on the two sides of the plate; but that as the bacterial suspension becomes thinner the colonies become fewer on the gentian violet agar and finally disappear entirely (plates B and C). Compare with Figs. 1 and 2.

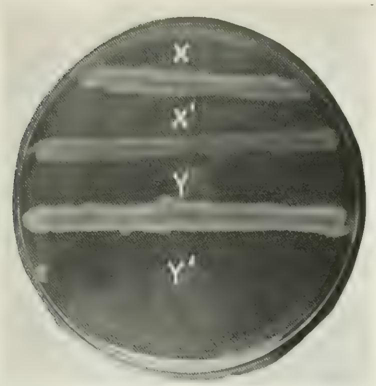


FIG. 9.—Plain agar plate stroked with strains *X* and *Y* to show the effect on them of exposure to gentian violet. *X*, Strain *X* unstained for control. *X'*, Strain *X* which has been stained with gen. vi.; *Y*, Strain *Y* unstained for control; *Y'*, Strain *Y* which has been stained with gen. vi.

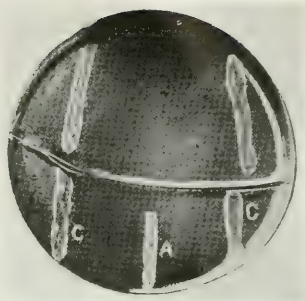


FIG. 12.—Persistence of a Gram-negative organism in a wound treated with gentian violet. The organism obtained from the wound has been stroked across the plate at the sides (*C*). *A* is a stroke of *M. aureus*, for contrast.

FIG. 10.—Experiment to show that a dye chemically unrelated to the tri-phenyl-methanes may also possess the selective bacteriostatic power. Upper half of the plate contains agar to which bismarck brown has been added; this is an azo-dye. *C*, *B. coli*; *T*, *B. typhosus*; *S*, *B. subtilis*; *A*, *aureus*.

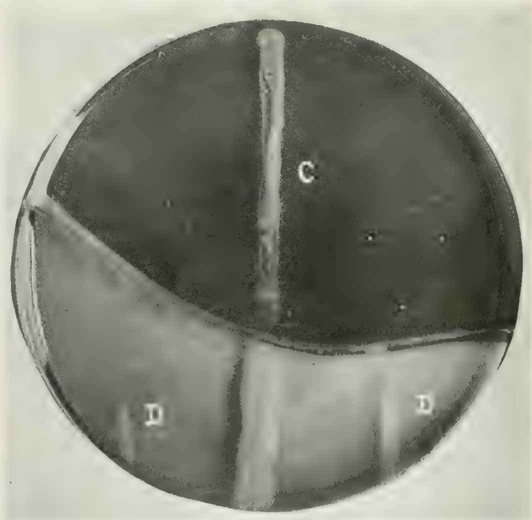
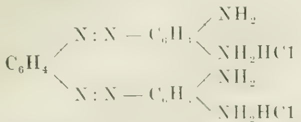


FIG. 11.—Effect of gentian violet on *B. diphtheriæ*. Divided plate stroked with this organism (*D*), and with *B. coli* (*C*) for contrast.

(4) But there are other considerations which warrant the belief that the chemical affinity hypothesis is not the correct one, or at least that chemical affinity is not the only factor involved. It was at first believed that the selective bacteriostatic power manifested by gentian violet and other members of the tri-phenyl-methane series was peculiar to this group. Most members of the group examined exhibited it (Fig. 5), while most of the dyes belonging to other chemical groups did not. The experience with bismarck brown, however, showed that in drawing the conclusion that the selective power was limited to the tri-phenyl-methane group, we had fallen into an error which the previous experiments had given no cause to suspect. This dye does not belong to the tri-phenyl-methane series. It is an azo-compound and has the formula:



In the early experiments there had been no evidence that it possessed any selective bacteriostatic power. It was found later, however, that, by varying the amount of bismarck brown used in the media, a dilution could be reached at which this dye, too, prevented the growth of Gram-positives, while without effect on the Gram-negatives (Fig. 10).

Although the fact has never been brought out by those who have written on the bactericidal power of the flavines, it is probable that these dyes too are less effective against the Gram-negative organisms than they are against the Gram-positives. In one of the few careful bacteriological studies, made during the war, of wounds which had been treated with these dyes Bashford[5] mentions with surprise the fact that, although in wounds treated with Dakin's solution it is the cocci which disappear last, in wounds treated with flavine the commonest organism to outlive others on the wound surface is a Gram-negative bacillus.

(5) A recent study by Kämmerer[6] has brought still more convincing evidence against the hypothesis of specific affinity between chemical group in the tri-phenyl-methane molecule and the Gram-positive organisms to account for the selective activity of these substances. This observer has shown that an exactly similar selective bacteriostatic power is possessed by mesohematin and certain metallic compounds of mesoporphyrin (*e. g.,* compounds with manganese and magnesium).

Mesohematin, when added in very weak solutions to the media on which organisms have been planted, inhibits the Gram-positive organisms but is without effect on the growth of the Gram-negatives. This substance has the formula $C_{34}H_{36}O_4N_4FeOH$, and is quite unrelated structurally to the aniline dyes.

The ultimate explanation of the Gram reaction is not yet known, but the trend of present experimental study is in the direction of a mechanical rather than a chemical theory of the process. Kämmerer regards the greater susceptibility of Gram-positive organisms to mesohematin and to gentian violet as due to their greater permeability. In his opinion these substances really reach the interior of the positive organisms and thus kill them; the Gram-negatives they are only able to attack on the surface and they therefore do them no harm. For this conception there is a large amount of evidence. It is, however, somewhat difficult to make my own experiments with stained small groups (containing 30 individuals) of *B. coli* conform to it. These organisms were exposed to the dye under microscopic observation until they had become stained a deep black. It was hard to imagine that the dye was simply resting on the surface of the bacteria. Yet they grew luxuriantly when planted on agar.

While the mechanism of the Gram reaction, therefore, though probably not chemical in nature, is still the subject of some discussion, the important point which the gentian violet studies have brought out is that it is of clinical significance. Speaking generally the Gram-negative organisms are less susceptible to many, possibly to all, selective bacteriostatic substances than the Gram-positive organisms. General protoplasmic poisons will kill Gram-positives and Gram-negatives alike. But these will also kill, or greatly injure, tissue cells. Substances which act in selective fashion will be more likely to be effective against the Gram-positives than against the Gram-negatives; hence, the latter will be the more resistant organisms. To this rule there will be exceptions presented by the very delicate Gram-negatives, like the gonococcus, whose growth may be hindered by a variety of slightly unfavorable conditions in the environment; and by the very vigorous Gram-positives, like some of the spore-bearing anaërobes whose growth is inhibited only by strong solutions of gentian violet.

A study of attempts to sterilize infected amputation stumps with gentian violet emphasized the significance of the Gram reaction of the infecting organism, at least so far as gentian violet is concerned. This study has been reported elsewhere.[7] Stumps infected with the Gram-positive *B. diphtheriæ* were rather easily freed of this organism; those infected with the Gram-negative *B. coli* could not be sterilized with gentian violet (Figs. 11 and 12).

[5] British Med. J., 1917, II, 849.

[6] Arch. f. Exp. Path. u. Pharm., LXXXVIII, Hft. 5 and 6, S. 247.

[7] Jour. Am. Med. Assn., 1920, Jan. 12, vol. 74, pp. 145-148.

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THE TREATMENT OF PLACENTA PRÆVIA, TOGETHER WITH THE ANATOMICAL DESCRIPTION OF TWO SPECIMENS

By William B. Thompson

(*From the Obstetrical Department of The Johns Hopkins Hospital and University*)

During the past few years a rapidly growing mass of literature has accumulated which advocates the adoption of more radical methods in the treatment of placenta prævia. Particularly since the appearance of Krönig's article upon the subject the employment of Cæsarean section has been recommended as the safest and most conservative method of treating selected cases of this serious complication of pregnancy and labor, and certain writers have even gone so far as to advocate its routine employment.

For the past decade, at least, Dr. Williams and his associates have maintained and put in practice the belief that except in rare instances conservative measures give better results so far as the mother is concerned than more radical operative interference. In order to determine whether the results obtained justify such a belief, I have gone over the first 10,000 admissions to the obstetrical ward of The Johns Hopkins Hospital and have analyzed the cases of placenta prævia included therein. I shall take this occasion to summarize the results of my investigation, and afterwards I shall describe in detail the anatomical findings in two specimens obtained from patients in the series, which will be illustrated by drawings kindly made by Mr. Max Broedel.

TABLE I.—SHOWING INCIDENCE OF PLACENTA PRÆVIA

	Admissions	Number of prævia	Percentage of prævia
Full term deliveries	7,752	33	50
Premature deliveries	635	21	32
Duration of pregnancy not stated.	3	3	4.5
Abortions	651	8	12
Died before delivery	15	1	1.5
Discharged before delivery	657	..	..
Admitted post partum	208	..	..
Not pregnant	79	..	..
Total	10,000	66	100

Table I shows that 10,000 women were admitted to the ward from its opening in 1896 to July 31, 1919, of whom 9054 were delivered at or before full term.

In the series 66 cases of placenta prævia were noted—an incidence of 1 to 137, or 0.73 per cent. Such a figure, however, gives too high an idea of the frequency of the complication; for during the same period an approximately equal number of patients were cared for in the out-patient service, and were sent into the hospital for treatment, whenever serious complications arose. Accordingly, our figures must be divided by two, which gives an incidence of 1 to 274, or 0.37 per cent. Even this figure is still too high, as many of the patients were not originally cared for in the service, but were referred to it after the midwife or physician in attendance had recognized the seriousness of the condition, as well as their inability to treat it properly. Taking these facts into consideration it would seem conservative to estimate that placenta prævia does not occur oftener than once in 400 pregnant women, and therefore may be regarded as a comparatively rare complication.

While it may be noted from Table I that 50 per cent of the cases occurred in full-time deliveries, closer consideration of the figures will show that the complication occurs relatively more frequently under other conditions. Thus, upon taking into consideration only the patients admitted to the ward, it is found that in full term deliveries the incidence was 1 to 235, or 0.43 per cent, as compared with 1 to 30, or 3.31 per cent, at premature labor. In other words, our figures indicate that placenta prævia occurs nearly eight times more frequently at premature than at full time labor, although it must be admitted that such an estimate is only approximately correct.

In view of the relatively large number of colored patients in the service, it may be interesting to ascertain whether the racial factor appears to have any influence upon the incidence of placenta prævia, and Table II gives information upon this point, as well as upon the influence exerted by the multiparity of the patients.

TABLE II.—SHOWING INFLUENCE OF RACE AND MULTIPARITY

	White	Black	Total		White	Black	Total
1—para	6	2	8	10—para	1	2	3
2—para	6	1	7	11—para	1	0	1
3—para	6	3	9	12—para	1	0	1
4—para	6	2	8	13—para	1	1	2
5—para	7	1	8	16—para	1	0	1
6—para	5	2	7	17—para	1	0	1
7—para	3	0	3	Not stated	2	1	3
8—para	0	1	1	Total	48 or 72.7%	18 or 27.3%	66
9—para	2	1	3				

As approximately 45 per cent of the patients admitted to the service are black, one should expect, roughly speaking, that a similar proportion of placenta prævia cases should be noted in that race. Yet, our figures indicate that the incidence is

only about two thirds of the calculated expectancy. Whether this discrepancy is to be attributed to the relatively small number of cases with which we have to deal, and is therefore only apparent, or whether it is due to some definitely racial influence cannot be decided until more extensive observations are available. A point which speaks in favor of the latter view is that if multiparity and the existence of endometritis play the part usually assigned to them in the etiology of placenta prævia that complication should occur more frequently in the black than in the white race, on account of the unusual prevalence of such conditions in the former. As our figures, however, show that placenta prævia occurs less frequently, a certain presumption is raised in favor of some racial influence—a point to which attention has not previously been directed.

Table II likewise demonstrates the influence of repeated child-bearing in the production of the abnormality, and abundantly confirms the well known observation that primiparæ are much less liable to placenta prævia than multiparæ. As forty per cent of our patients enter the service to have their first child, approximately 26 of our 66 placenta prævia patients should have been primiparæ, but our figures show that only eight of them were.

Analysis of the figures also shows that the multiparous women had on the average a much larger number of children within a short time than normal. Thus, the 55 multiparæ concerned averaged 5.9 children each in 9.7 years from the date of the first delivery, which greatly exceeds the fecundity of the average married woman, and lends further support to the generally accepted view that rapidly recurring pregnancies favor the development of disturbances of the endometrium, which in turn predispose towards abnormal implantation of the placenta.

TABLE III.—SHOWING THE AGE OF THE PATIENTS

Age	White	Black	Total
16–20 years	4	5	9
21–25 years	11	3	14
26–30 years	11	3	14
31–35 years	9	5	14
36–40 years	9	0	9
41 years on	2	1	3
Not stated	2	1	3
Total	48	18	66

Table III analyzes the ages of the patients when treated for placenta prævia. Naturally, it brings out the fact that the complication occurs most frequently during the most active period of child bearing. Possibly, the only two points of interest shown are that a comparatively large number of women present the abnormality before they have passed their twentieth year, and, on the contrary, that very few women near the menopause are involved. It does not, however, seem permissible to attempt to draw any definite conclusions from so relatively few cases.

Table IV gives the relative frequency with which the various types of placenta prævia were noted in the series, as well as the percentage incidence of each, and also indicates the number of maternal deaths. Our figures are in accord with those of most writers upon the subject and show that partial placenta prævia is the most usual variety, while the central type occurs much less frequently. Attention should be directed to a relative decrease in the number of centralis cases diagnosticated during recent years. This is not the result of any actual diminution in the number of such cases, but is due to greater accuracy in diagnosis. It would appear that in the early years of the service the diagnosis of placenta prævia centralis was sometimes based upon finding the region of the internal os entirely covered by placental tissue when the cervix was only sufficiently dilated to permit the introduction of one or two fingers. Further experience has demonstrated, however, that a diagnosis made at that time is not always correct, as it has sometimes happened in such cases that examination after the cervix had become fully dilated has shown that it was only partially covered by placental tissue, so that what had appeared as a central placenta prævia at the first examination was in reality only a partial one.

TABLE IV.—SHOWING THE FREQUENCY OF THE SEVERAL TYPES OF PLACENTA PRÆVIA AND THE MATERNAL AND FŒTAL MORTALITY IN EACH

Type	No. cases	Percentage	Maternal deaths	Fœtal deaths
Centralis	14	21.21%	4	13
Partialis	27	40.91%	2	21
Marginalis	24	36.36%	0	13
Lateralis	1	1.52%	1	1
Total	66	100.%	7	48

Stress should be laid upon this point, and the diagnosis of the central type should not be made unless the cervix is fully dilated, or, unless the finger introduced through the partially dilated internal os can feel placental tissue extending sufficiently far beyond its periphery to insure that it will still cover it when dilatation is complete. Such a restriction is particularly necessary at this time, for the reason that not a few writers advocate very radical treatment in the central type of implantation.

As was indicated in Table IV, seven of the 66 mothers were lost—a mortality of 10.6 per cent. At first glance this seems abnormally high, but when allowance is made for the fact that a large proportion of the women were not admitted until late in labor and after profuse hæmorrhage and extensive manipulation, the results, while leaving something to be desired, are not bad.

The complication was treated by various methods, and while the number of cases is too small to permit the drawing of absolute conclusions, the experience of the service, nevertheless, definitely indicates that some procedures are much safer than others. Table V gives a synopsis of the methods of treatment

TABLE V.—SHOWING METHODS OF TREATMENT

Method of treatment	Cases	Maternal deaths
a. Died undelivered....................	1	1
b. Braxton Hicks' version	2	1
c. Rupture of membranes..............	2	0
d. Cæsarean section..................	2	1
e. Admitted in 2d stage	8	2
f. Manual dilatation of cervix.........	15	2
g. Ballon	36	0
Total............................	66	7

employed, together with the number of maternal deaths associated with each. Each method will be considered briefly, after which an attempt will be made to decide which is the safest and the most dangerous, respectively.

The case of the patient who died undelivered will be described in more detail at the end of the article (Case I), and is of especial interest, as her death was the only one in the series following the use of the ballon. Moreover, it is particularly interesting, because the excellent drawing accompanying the description gives graphic evidence of how slight a degree of placental separation may lead to fatal hæmorrhage.

The two patients who were treated by Braxton Hicks' bipolar version followed by slow extraction presented the partial and marginal type of placenta prævia, respectively. One died as the result of excessive loss of blood, and the other recovered. Such results are entirely accidental and do not give a fair idea of the merits of the procedure, which in general should be regarded as the best treatment when rubber ballons are not available.

In two other patients with marginal placenta prævia, rupture of the membranes sufficed to check further separation of the placenta, while prompt engagement of the presenting part controlled the bleeding and allowed labor to progress spontaneously. Doubtless this method of treatment, which is so popular in France, might have been employed advantageously in other marginalis cases.

The two instances in which Cæsarean section was employed require especial mention. Both were examples of complete placenta prævia. The first occurred in a 40-year-old primipara, who was also suffering from chronic nephritis, while the second constituted an accidental finding in a uterus which was removed by supravaginal amputation on account of atresia of the cervix and intra-partum infection. The latter will be described in detail as Case II, and the excellent drawing accompanying it will afford an ideal illustration of a complete placenta prævia *in situ*.

In the first patient, the condition was complicated by a rigid primiparous cervix and an inelastic pelvic floor, so that, although the child was dead at the time of admission, it was felt that Cæsarean section offered the most conservative method of delivery. The patient died on the eighth day, and autopsy showed that death had resulted not from infection, but from an exacerbation of the nephritis from which she had suffered for years. This case is of further interest, as it represents the only one in the series which afforded Dr. Williams and his associates an indication for treating placenta prævia by this means. Naturally, a single experience cannot be utilized as an argument either for or against any method of treatment, but the case may be utilized as showing how rarely one encounters what is regarded here as a valid indication for the employment of Cæsarean section—the association of a rigid cervix with placenta prævia.

Eight patients were admitted in the second stage of labor, and as dilatation of the cervix had already taken place spontaneously, all that remained was to effect delivery by the most conservative means available, such as forceps, version and extraction, etc. It is interesting to note that in three of these cases the child presented transversely, and in one of them the shoulder was so impacted and the uterus in such tetanic contraction that version was out of the question and delivery was effected after decapitation. This patient died, as well as one other, who was admitted after a long course of maltreatment outside. The latter presented a rhachitic pelvis which was so contracted that a Cæsarean section would have been imperatively indicated had she been in good condition; but as the child was dead and the mother was profoundly shocked as the result of loss of blood and fruitless attempts at delivery, and furthermore as signs of intra-partum infection were present, delivery by craniotomy was effected as the last resource; the patient, however, died shortly after her return to bed.

It must be admitted, I think, that in neither of these instances can the fatal issue be attributed to the underlying condition, nor to the treatment employed in the service, as both are examples of the type of neglected cases which unfortunately are met with in all clinics.

Passing on to the group of 15 cases in which delivery was effected after manual dilatation of the cervix, the following may be said. In the early years of the service *accouchement forcé* was the routine method of delivery whenever the cervix was not sufficiently dilated to permit prompt termination of labor by the ordinary obstetrical procedures. In such circumstances the cervix was dilated manually by Harris's method, after which delivery was effected by version and extraction, with the exception of a single case in which the existence of a primary breech presentation made version unnecessary.

In this group we had to deal with the following varieties of placenta prævia—6 centralis, 4 partialis and 5 marginalis—and 2 mothers and 11 children were lost. In one of the fatal cases, the patient was moribund on admission, and would

probably have died irrespective of the method of treatment, so that her death can scarcely be attributed to the means by which delivery was effected. On the other hand, the other death was directly attributable to the operation. In this instance, which was reported to the American Gynecological Society by Dr. Williams, in 1906, the markedly exsanguinated multiparous patient was admitted with complete placenta prævia and the cervix somewhat less than half dilated. While Dr. Williams was examining her, profuse hæmorrhage occurred and the cervix seemed so soft that he felt that it could readily be dilated. Manual dilatation was effected with the greatest ease, the child turned and extracted and the placenta expressed. The cervix was then inspected and a deep tear extending to the fornix was discovered on the left side. This was exposed and apparently satisfactorily repaired, the patient being put to bed in good condition. Without further external bleeding, she soon began to do badly and died four hours later. At autopsy it was found that the tear had involved the lower uterine segment, and that only its lower portion had been repaired by the vaginal sutures, with the result that the bleeding had continued between the folds of the left broad ligament and had led to the formation of a hematoma 15 to 18 cm. in diameter.

Although members of the staff had long realized that manual dilatation of the cervix in placenta prævia was usually accomplished at the expense of deep cervical tears, which frequently required immediate repair, the fatality just described still more forcibly accentuated the dangers of the procedure, and led to its abandonment as a routine practice, together with the recognition that generally speaking it represents the most dangerous method of treating this complication.

Following the recognition of the danger of *accouchement forcé,* the use of the rubber ballon has in recent years become the routine method of treating placenta prævia except when the cervix is fully dilated when the patient is admitted to the service. Prior to the World War the reinforced Champetier de Ribes' ballon was employed with the greatest satisfaction, but since that time we have been forced to use Vorhees' bags instead. These are of domestic manufacture, and leave a great deal to be desired, in that they are less efficient as dilating wedges, and are distinctly less durable.

Ballons have been employed in 37 cases, 36 of which will be considered under this heading, while the remaining one will be described below as Case I. In the series the following types of placenta prævia were noted—6 centralis, 17 partialis and 14 marginalis. All of these patients recovered.

Ordinarily, the ballon is introduced into the uterus after rupturing the membranes, or after perforating the placenta in centralis cases, in preference to the extraovular method advocated by Kosmak and others, because it has been felt that better results are obtained by compressing the placenta against the area of separation. Where haste was essential, traction was made upon the bag by means of a weight attached to the end of the tube and allowed to hang over the foot of the bed. Usually the largest-sized bag was allowed to remain in place until it was expelled spontaneously, after which the course pursued varied according to circumstances. If the bleeding had ceased, and the patient was in good condition spontaneous delivery was awaited, and occurred in 10 of the 36 cases. On the other hand, if the bleeding continued, or if the patient was in poor condition or if the uterine contractions appeared inefficient, delivery was effected by the most conservative means available, the records show that version and extraction were employed in 18, breech extraction in 5, and forceps in 3 cases.

More particularly since we have been compelled to employ Vorhees' bags, complete dilatation of the cervix has not always been effected, so that in 5 instances it has been necessary to complete the process by manual methods. These, however, are not comparable with the *accouchement forcé* which was formerly employed, since, as the major part of the dilatation had already been effected by the ballon, all that is required is to overcome the final resistance. Such secondary manual dilatation was required three times on account of premature and repeated rupture of the ballon, and twice on account of tetanic uterine contractions, or because the patient's condition was so serious that it was felt that she could not survive a longer delay.

As has already been indicated, all of the 36 patients in this series recovered, and such a result is the more remarkable, as they were not treated by a single individual possessing unusual dexterity and judgment, but by a succession of resident obstetricians who naturally varied considerably in ability. With such maternal results, it can justly be claimed that that treatment by means of the ballon is eminently satisfactory, and accordingly one can readily understand why the employment of Cæsarean section has found so little favor in this service.

Of course, it must be admitted that the latter affords a ready method of coping with the situation, and does not require the hours of waiting incident to more conservative methods of treatment. On the other hand, it must be borne in mind that many of the patients, who are admitted exsanguinated and after having been subjected to vaginal manipulations by physicians with imperfect technique, are poor surgical risks, so that the mortality following Cæsarean section in them must inevitably exceed that noted in our service. Furthermore, it should be remembered that the uterine cicatrix following Cæsarean section represents a *locus minoris resistentiæ,* so that the possibility of its rupture in subsequent pregnancies constitutes a danger, which certain writers consider so great as to justify the dictum "once a Cæsarean always a Cæsarean." Consequently, if a Cæsarean is done, it may condemn the patient to repeated sections in any future pregnancies. If this is the case, the unprejudiced observer must admit that it is a questionable procedure so to treat the ordinary case of placenta prævia that this will be necessary, more particularly as other methods of treatment are available, which give as good or even better immediate results and do not compromise the future child-bearing career of the patient.

On the other hand, it is argued by the advocates of radical treatment that Cæsarean section offers additional advantages in the way of better prospects for the child, and at first glance such an argument appears convincing, more particularly when

we admit that less than 30 per cent of the children in our service were saved. It should, however, be remembered that placenta prævia is a complication which tends to become manifest at a period when the child's prospects are minimal. Our figures show that it occurs eight times less frequently at term than at premature labor, so that we ordinarily have to deal with children which have no chance for continued existence after birth, or which are so premature that they readily succumb to the loss of blood or other conditions incident to the condition. Consequently, it seems justifiable to demand that Cæsarean section should be employed only under the strictest indications, and should not be considered as a routine method of treatment. In addition, it should be realized, that placenta prævia is inevitably associated with a high infantile mortality, and consequently obstetricians should hesitate to advocate methods of treatment which, while they may save the lives of a few more children, do so at the expense of increased maternal mortality.

I shall now briefly describe two cases in the series, from which we were fortunate enough to obtain, at autopsy, specimens which throw important light upon some of the anatomical features of placenta prævia, and whose value is greatly enhanced by the fact that Mr. Max Broedel was kind enough to prepare drawings illustrating certain salient points.

Case I.—H. 9795. The 21-year-old patient had previously had two spontaneous labors. She registered in the out-patient service and one week later had a severe hæmorrhage, but did not call the service until she was markedly exsanguinated. She was admitted to the hospital *in extremis,* and presented the characteristic symptoms of acute anæmia.

Examination showed that the pregnancy was practically at term and that feeble uterine contractions occurred from time to time. The external os was 3 to 4 cm. in diameter, with placental tissue covering the internal os. A large-sized bag was immediately introduced, a salt solution infusion begun, and various stimulants administered. Although not more than 200 c. c. of blood was lost, the patient died undelivered three hours after admission.

The ballon was removed after death and the autopsy gave negative results, except for the signs of severe secondary anæmia. The pregnant uterus was removed unopened and placed in formalin. After hardening, it was opened by a sagittal median section, when it was found that we had to deal with a placenta prævia partialis, which was inserted upon the posterior wall of the uterus. The placenta measured 16 x19 cm.; it varied in thickness from 1.5 cm. at its upper part to 3.5 cm. at its lower pole, and extended from the internal os two-thirds of the distance to the fundus. Fig. 1 shows that it was firmly attached to the uterine wall, except for a distance of 2 cm. just above the internal os, where it had been separated from its attachment, and it was from this area of detachment that the fatal hæmorrhage had occurred. The cervical canal was intact, measured 4 cm. in length, and presented the usual rugous appearance with a clear mucous secretion.

Examination of microscopic sections through the cervix showed no abnormality except a moderate leukocytic infiltration. Sections through the uterine wall and placenta above the area of separation showed that the placenta was typically implanted upon a thin decidua basalis; while sections through the area of separation showed that the detachment had occurred in the spongy layer, the greater part of the decidua being attached to the detached portion of the placenta, while only a very thin layer remained in contact with the muscularis. The decidua covering the detached pole was moderately infiltrated with leukocytes, particularly at its lowermost extremity. At the upper angle of the area of separation, a large vein had been torn through, and readily explains the origin of the fatal hæmorrhage.

This specimen is of interest from two points of view. It shows, in the first place, how small an area of detachment may lead to a fatal termination; while in the second place it represents the only fatality in the series following the use of the rubber ballon. As the patient was practically moribund when admitted, her death can not be ascribed to the treatment pursued, as the result would have been the same whatever might have been done.

Case II is of interest particularly because it affords an opportunity to study and portray a central placenta prævia *in situ.*

Case II.—H. 5381. The 35-year-old patient was sent to the hospital by her physician on April 27, 1912, with the statement that she had been in labor three days, had no cervix, and required operative delivery. The patient stated that she had had three spontaneous labors, the last six years previously, and one miscarriage two years later. Shortly after the miscarriage she was operated upon at another hospital when her cervix was amputated, the right ovary removed, and the uterus suspended. A year later she was operated upon at a second hospital when the right tube and part of the left ovary were removed, and numerous adhesions freed. At that time the uterus was found to be firmly fixed to the anterior abdominal wall, but the fixation ligament was not interfered with.

On admission to the hospital the temperature was 100.6° F. and the pulse 85. On examination the pelvis was found to be normal, the fundus reached midway between the umbilicus and ziphoid, and was so tetanically contracted that the outlines of the foetus could not be distinguished. Fœtal heart sounds were not heard. A pigmented scar, 12 cm. long, occupied the midline of the lower abdomen. Palpation showed that the round ligaments diverged upwards, and that a softish flattened structure, the size of a finger, lay to the left of the abdominal scar and was thought to be adherent intestine. On vaginal examination no trace of the cervix could be found, but the vault of the vagina was occupied by a nodular area which was marked by several radiating depressions extending from a common center. Examination with a speculum gave similar results. In view of the condition of the cervix radical interference seemed necessary, and it was debated whether this should consist in Cæsarean section or in a vaginal incision through the cervical region. In view of the existence of intra-partum infection and the fact that the child was already dead, it was determined to remove the unopened uterus by deep supravaginal amputation. At the same time it was recognized that the operation would probably be complicated by the fixation of the uterus, as well as by intestinal adhesions.

After the usual preparation the old scar was excised and the abdominal cautiously opened in the midline. The adherent intestine, which had previously been palpated, extended parallel to the incision for a distance of 6 to 8 cm. on the left side. A second loop of intestine was likewise adherent to the abdominal wall and extended at a right angle across the incision. This was peeled off and the incision was extended above the umbilicus. The elongated fixation ligament then came into view and was represented by a band 4 mm. in diameter extending from just below the lower extremity of the abdominal incision to the anterior surface of the uterus just beneath the fundus. After this had been doubly ligated, the uterus was eviscerated when it was found that the right ovary was absent, while the corresponding tube was 4 to 5 cm. in length and presented a patent fimbriated extremity. The left tube, which was converted into a hydrosalpinx, was densely adherent to the uterus, as well as to a loop of intestine at its distal end.

The uterus was then amputated unopened as low down through the cervix as possible. This was comparatively difficult, owing to the fact that the lower uterine segment was distended by a bulky portion of the fœtus. In the center of the cervical stump a small conical depres-

FIG. 1.—Sagittal section through lower and posterior portion of the uterus and cervix from a patient dying undelivered ($\times\frac{2}{3}$). It shows a partial placenta prævia in situ, and demonstrates how small an area of separation may lead to fatal hemorrhage.

FIG. 2.—Sagittal section through a full term pregnant uterus removed unopened by supravaginal amputation ($\times\frac{2}{3}$). It shows a central placenta prævia *in situ* with velamentous insertion of the umbilical cord. The tag of tissue on the upper part of the anterior wall represents the proximal end of the false ligament resulting from the previous fixation. Note the cup-like form of the placenta, and its variations in thickness in different locations.

sion, apparently lined with mucous membrane, was noted, which was excised by a funnel-shaped incision. The stump was closed by interrupted catgut sutures, after which the broad ligament wounds were united in the usual manner. The adherent loops of intestine were then freed from the abdominal wall and the incision closed in four layers. None of the uterine contents escaped into the abdomen, and very little blood was lost. The recovery was very satisfactory, the highest temperature being 101.5° F. on the tenth day, and the patient was discharged on the twenty-fourth day. At that time the cervix was represented by a few nodulations in the vaginal vault, but no trace of the external os could be discovered.

Description of Specimen.—Immediately after removal, the uterus was placed in formalin, and after hardening formed a pyriform mass, 26 x 22 x 18 cm. In the midline of its anterior surface, 6 cm. below the fundus, is the uterine end of the fixation ligament, while the uterine attachments of the round ligaments are at a slightly higher level and are 19 cm. apart. The right tube is apparently normal, except for a glistening cystic structure, 1.5 cm. in diameter, on its anterior surface; the left tube is covered by adhesions. The left ovarian ligament is so thinned out as to resemble a peritoneal fold, and at its free end is an elongated mass of tissue, 2.6 x 6 x 7 cm., which probably represents the ovary.

At the site of the cervical amputation is an opening, 6 cm. in diameter, which is occupied by dark spongy tissue resembling the placenta in appearance. This is firmly adherent posteriorly and to the right, but is free anteriorly and to the left. Upon opening the uterus by a sagittal median incision a large macerated male child was found in the right acromio-dorso-anterior position, the left shoulder being firmly impacted in the lowermost part of the uterus. Upon removing the child, it was found that we had to deal with a placenta prævia centralis, which filled out the entire uterine segment like a cup. It measured 8.5 cm. on the posterior and 12 cm. on the anterior wall of the uterus, and was much thicker posteriorly than anteriorly, measuring 1.5 and 0.7 cm., respectively. Corresponding to the line of amputation through the cervix the apex of the placental cup is very thin and does not exceed 3 mm. in thickness. Coursing over the inner surface of the anterior portion of the placenta are many large vessels, which extend upward to join the cord (Fig. 2).

Figure 2 shows very clearly that the major portion of the placenta is firmly united to the uterine walls, except for a distance of 5 cm. on the anterior and 4 cm. on the posterior wall of the lower uterine segment, where a small space exists. The umbilical cord is inserted velamentously upon the anterior wall of the uterus 2 cm. above the upper margin of the placenta and large vessels make their way downward through the membranes toward the latter. Generally speaking the placenta appears smaller than the average, and on section presents a pale dense appearance so that, except for a small portion, it seems to be devoid of blood.

The uterine walls vary from 11 to 3 mm. in thickness, being thinnest in the lower uterine segment. There is no indication of a contraction ring, and the protrusion noted on the posterior wall probably resulted from pressure against the promontory of the sacrum.

Microscopic Examination.—Sections through the uterine wall above the placental site show that the amniotic epithelium has become desquamated and that the chorionic membrane is represented by a layer of clear connective tissue from which the nuclei have almost entirely disappeared. The decidua vera is in great part normal. It is made up of small decidual cells and contains a few flattened glands. At its junction with the chorionic membrane there are numerous areas of closely packed polymorphonuclear leukocytes in various stages of preservation, which apparently represent minute miliary abscesses. The muscularis is normal and its fibers in great part follow a parallel course.

Sections through the posterior wall at the placental site show that the placenta is almost entirely infarcted and presents numerous areas of calcification. No trace of decidua is seen beneath it, for the reason that the tissue is so densely infiltrated with leukocytes as to obscure all details of structure. The leukocytes extend far out into the muscularis, forming parallel rows between its fibers. Sections through the upper part of the placental site on the anterior wall show an identical appearance, but lower down the placenta is separated from the anterior wall of the uterus by a narrow space. The free surface of the placenta is covered by a layer of tissue which is so densely infiltrated with leukocytes that it is impossible to determine its histological character; while the corresponding uterine wall is acutely inflamed and the leukocytic infiltration extends through the entire thickness of the muscle, and has so obliterated all structure that it is impossible to say whether its internal surface had been covered by decidua or by cervical mucosa, although its corrugated appearance and the presence of a few irregular glandular spaces make it probable that we have to deal with the latter. Furthermore, the free portion of the placenta below the site of implantation is covered by a membrane which is so acutely inflamed as to obscure all details of structure.

This specimen is of great interest from three points of view. First, as to the origin of the atresia; second, the mode by which the uterine infection occurred; and third, the abnormal implantation of the placenta. Naturally, the first thought would be that the atresia had followed the high amputation of the cervix four years previously, but the fact that the patient had continued to menstruate regularly up to the onset of the present pregnancy clearly indicates that the complete atresia could not have existed; moreover, if it had, impregnation could not have occurred.

Concerning the origin of the uterine infection, it can only be said, that as the inflammatory reaction is so intense and involves the entire interior of the uterus, it is improbable that it is of recent origin. For this reason, it seems permissible to assume that a moderate atresia had followed the operation, but had not been sufficiently pronounced to prevent the escape of the menstrual discharge or to interfere with conception, and that some time after the occurrence of pregnancy the patient had attempted to induce abortion; that this had not had the desired result, but had led to inflammatory changes, which resulted in the complete closure of the atresia and the infection of the interior of the uterus. Against such a supposition is the fact that the patient positively denied any such attempt.

The velamentous insertion of the cord permits speculation as to the cause of the abnormal implantation of the placenta. From what we know of the ordinary processes of implantation, it may be assumed that the point of origin of the uterine end of the cord corresponds to the point of attachment of the abdominal pedicle in early pregnancy, which in turn corresponds to the portion of the chorion which later forms the placenta. For this reason it may be assumed that the ovum was originally implanted upon the middle of the anterior wall of the uterus, but for some reason the chorion frondosum found the tissues in that location little adapted for implantation and consequently the placenta was developed lower down, and, as sufficient room was not available for it on the anterior surface of the uterus, it extended over the internal os to the posterior surface, thus giving rise to the prævial condition.

ON THE SUPPOSED LIFE-CYCLE OF BACTERIA*

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Most diseases from which mankind suffers are due to parasites, many of which, for instance, Treponema pallidum, under natural conditions, are unable to live outside the human body. On the other hand, some of them have an independent life, as saprophytes or parasites outside of the body, and only occasionally become pathogenic. Bacillus tetani and actinomyces may be cited as examples of this kind. A third group of pathogenic organisms have a regular life-cycle, so that a part of their development takes place inside, another part outside the human body. The malarial organism is the classical paradigm for this group.

Concerning bacteria, the general consensus of opinion has been that they have no life-cycle of this kind. Their morphology has been considered to be relatively simple. But during the last decade many scientists have attacked this problem from different points of view and reached conclusions differing from the usual opinion. The first to emphasize this idea against the popular theory was Ernst Almquist, who as early as 1884 pointed out, in disagreement with Koch, that the epidemiology of typhoid fever could not be explained on the assumption that the virus was produced only in the human body. According to Almquist, the typhoid bacillus develops a saprophytic stage outside the body, during which it not only propagates but even acquires new and important characteristics.

The basis for these ideas was that the theory adopted by Koch could not explain why the epidemics appeared at certain times of the year and did not account for the peculiarities shown by the curve of morbidity during epidemics caused by infection through the water supply.

In this connection, you can recall some other peculiar epidemiological facts hitherto unexplained, for instance, the changing virulence during different epidemics, or the problem how in some diseases—influenza, for example—the pathogenic organism survives from one epidemic to another. Now, if it is possible that a life-cycle takes place outside the human body, it may be considered likely that such may occur in the body itself. The difficulty in demonstrating the specific organism in certain disturbances caused by syphilis or tuberculosis has brought many to the idea that the microörganisms are here in a special unknown stage of development. Important records that could support the theory of a cyclic development of certain bacteria inside the human body are given by Hort and Mellon. I shall refer to these later.

It might be best first to consider the original investigations of Almquist in order to understand better the somewhat discordant results of later authors on this subject. According to Almquist, the typhoid bacillus grown on drying agar at low temperature does not propagate in the usual way by simple division but in the following special way. Coccoid bodies are developed from the rods by budding. These coccoid bodies are of two kinds: first, irregular protoplasmatic masses, and, second, sharply defined round ones with thick walls. From both kinds, rods or filaments can grow later and they in their turn produce new coccoid bodies. The different cells are kept together for a long while by fine connecting threads. The filaments can be very numerous and form an intricate system, which was called by Almquist the "myceloid." To the protoplasmatic masses he gave the name "bacteria plasmodia." Regarding the thick-walled bodies, Almquist made the surprising statement that they contained several separate small cells that were liberated by the bursting of the shell.

These bodies which bud from rods or coccoids, Almquist called exogenic bodies or bacterial conidia, but did not describe the more particular connection between the different forms. Those developed at low temperature had no ability to move and some of them showed an increased virulence, while those exposed to high temperature lost their virulence.

The question of virulence was taken up for further study by the collaborators of Almquist—Korén and Olsson, who primarily used Löffler's bacillus and Vibrio choleræ for their investigations. The first of these organisms when grown on a special medium at low temperature produces small needles measuring one or two microns. These are avirulent, but when transferred to the usual media they regress to the normal form of the bacillus, regaining their virulence.

The investigations of Olsson on Vibrio choleræ are more elaborate. He found that the vibrios, when grown for a longer time on soil extract, lost their ability to move and grew longer in shape. When these were transferred to agar, they produced shorter, still motionless, and less virulent forms. These peculiarities were visible through a number of generations, but after a while the vibrios returned to the original normal form, but showed a toxicity up to ten times stronger than in the original cultures.

Other observations made by Almquist are worth mentioning. In filtering about a hundred typhoid cultures he found that in ten of these there was to be seen a very small coccoid microorganism, which was most difficult to grow and which was entirely different from the typhoid bacillus. At the same time, however, this aberrant form was agglutinated by typhoid serum in a dilution of 1 to 500 and produced, when injected into rabbits, antiserum for typhoid. Almquist suggested that this form might be a "mutation" and because of the fact that it produced antiserum for typhoid he has given it the peculiar name Bacterium antityphosum. Almquist found other small immovable forms by examining blood from typhoid patients in the hanging drop. He makes no attempt to explain these different phenomena in referring the bacteria to other groups of microörganisms. He only remarks that the plasmodia remind him of similar structures in the myxomycetes

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and that the conidia are not unlike the bodies of this same name in the group of the fungi.

The problem of the systematic position of the bacteria was early considered by Sopp. He holds the opinion that the bacteria are really a kind of fungi. His view has been supported by A. Meyer, who in a long series of papers has demonstrated the striking resemblance between the structure of the cells in these two groups. During the four years in which I have been occupied in studying problems of this kind I have been brought to hold a similar view, that the bacteria are *fungi imperfecti*. I shall first try to show how far such a theory explains the above-mentioned facts discovered by Almquist and others. What are then the characteristics of such a fungus? Take as an example a monilia, and you will see that it propagates in two different ways—by budding and by simple fission. The buds can develop into short coccoids, to longer bodies like bacilli or to long filaments. The budding can occur at any point of the cell. The development of branches is substantially the same as in a building. On the other hand, both the short buds and the long filament can produce new cells by fission. If it is a filament that falls into a row of cells we speak of oïdia, and when buds arise on the side of a filament we call them conidia.

Sometimes you will find only one of these forms in a culture and sometimes several mixed together, according to the nature of the substrate and the age of the culture. If, for instance, you grow the uusual Oïdium albicans on agar you will only get coccoid forms, while you will find long forms and filaments if the culture is transferred to Löffler's serum. The filamental forms here become more and more pronounced during the series of generations. Investigations have shown that all these different forms can be found among nearly every kind of bacterium. If they do not appear on the usual substrates it is only necessary to change the chemical composition of the substrate or the temperature of the incubator. Striking pictures of these aberrant forms are to be found in the papers of Almquist, Sopp, De Negri, Hort, Meirowsky, Löhnis and Smith, Mellon, Wade and Manalang, Jones, Bergstrand, and others. The photographs given by Hort of bacteria of the typhoid group, and those of Treponema pallidum in the papers of Meirowsky are very clear.

Anyone can easily control these points by examining, for example, the Löffler's bacillus in the hanging drop. But it is absolutely necessary to avoid using dried and stained slides, for in a preparation like this every kind of artefact may interfere.

The objection that all these different forms depend upon contaminations is readily refuted, inasmuch as several of the authors named have used warm-stage methods and followed the development of the different cells. Furthermore, the forms in question occur in all cultures. To speak of contamination would mean to say that no pure cultures of bacteria exist. For this reason we need not consider the methods of Burri and Barber in making cultures from single cells.

In one of my papers I have given a review of the literature concerning different groups of Coccaceæ, Bacteriaceæ and Spirillaceæ, and have pointed out that they all behave in the same way with the exception of the spore-producing Bacteriaceæ which show an extraordinarily simple and uniform morphology.

Someone may ask if it would even be possible to grow staphylococci or meningococci so that they will change their form to rods. This is not possible—at least not at present—but Korén and Hort have demonstrated that they can reproduce by budding and this is the essential thing. In this connection compare the behavior of the different kinds of bacteria with that of various types of fungi. Saccharomyces is generally very stable and can only with difficulty be forced to change its form on various substrates, whereas Oïdium albicans can easily be grown as coccoid bodies or as filaments.

It is impossible in this short paper to compare in any detail the structure of the cells. This has been dealt with to a certain extent in some of my previous papers. To serve as an example from this field, I shall merely call attention to the fact that the polar granules of Löffler's bacillus are placed in a vacuole where they exhibit a dancing movement in just the same way as " die Tanzkörper " in the yeast cells. The theory will also probably throw light on such questions as Much's granules and the fragmentation spores of Boström.

So far it is easy to interpret the different forms of the bacteria by comparing them with fungi, but there are other forms that present considerable difficulties.

As we have said, Almquist found spherical forms of different kinds among typhoid bacilli. Most of these are probably just the usual vegetative cells, " short buds "; but others were thick-walled and these are apparently of another kind. These have been seen by quite a number of observers. In the diphtheria bacillus these walls show the characteristic that they can be stained with the Ziehl-Nielsen stain, and one would be likely to compare these with the durable cells as we find them in yeast. I have advocated this explanation and thus agree completely with Jones, who, in speaking of azotobacter, writes as follows:

> In cultures more than fourteen days old, large spherical, thick-walled cells are common. These appear to be resting cells or arthrospores. On transference to fresh media these thick-walled cells germinate, the cell plasma emerging from the thick wall as a large rod, which at once proceeds to multiply by fission.

Mellon's suggestion that these forms are possibly in some way connected with a rejuvenescence of the protoplasm must not be left out of consideration. Such processes are described by Woodruff in protozoa in which pure strains that are prevented from conjugation rejuvenate regularly by an intracellular process called endomixis. Even real conjugation, as in Saccharomyces, is said by Löhnis and Smith to occur among bacteria. These ideas may seem somewhat daring, but it is in fact most natural to look for some kind of rejuvenescence like that which occurs in other microörganisms.

Interesting and rather startling observations concerning these thick-walled cells have been published by Almquist, Hort and Mellon. They claim that these bodies contain within their walls a number of small cells that become free by bursting

of the wall. Mellon has seen these small cells move freely after liberation. Except for the mobility of the small cells there is here a striking resemblance to the sporulation of yeast cells, especially since the number of small cells within the wall seems to be fixed. In pictures shown to me by Mellon four small cells could be seen within each body. The supposed mobility of the small cells reminds one, on the other hand, of certain forms of protozoa. The importance of this mobility must not be exaggerated. We have seen that Bacillus typhosus and Vibrio choleræ can occur in mobile and immobile forms at the time when they show a typical fungus growth.

Jones has made somewhat similar discoveries working with Bacillus azotobacter. The only difference is that Jones did not observe the small cells inside spherical thick-walled bodies but within bodies of other shape. He writes:

> Following a period of reproduction by fission, individual cells develop reproductive granules or gonidia within their cell plasma, which on disintegration of the mother cell are dispersed, increase in size, become typical azotobacter short rods, ovals or spheres, and reproduce by fission.

We shall now consider the question of the nature of the bacteria plasmodia as described by Almquist, and the "symplastic stage" from the records of Löhnis and Smith, two probably corresponding features which we occasionally find mentioned also in very early literature. The two latter authors, especially, have considered this stage most important as the beginning and end of the life-cycle of bacteria. I can offer no suggestion as to the nature of these forms, whose importance also is advocated by Jones. I will only mention that I have seen similar structures in Löffler's bacillus when using India ink preparations, but little was to be seen in the hanging drop and stained slides; only gentian violet made such structures visible. But this stain is peculiar in that it stains the mucus that almost always occurs around old bacteria, so that it is difficult to recognize the shape and form of the bacterium itself. I was inclined to interpret these masses as clusters of degenerated bacteria kept together by mucus. Of course, it cannot be denied that in these masses there may be forms that are capable of reproduction.

It is not difficult to imagine that toxicity and virulence could be changed during such a supposed life-cycle, although it is not necessary to use such a life-cycle as an explanation for such changes. As Oïdium albicans shows such different morphological forms, depending upon the nature of the substrate, why should not changing environmental influences cause modifications in the physiological behavior of the organism? The investigations of Olsson on Vibrio choleræ show just this. Information given me by Rettger concerning experiences in his laboratory points in the same direction. This must not be taken to mean that a certain toxicity must be connected with a certain morphological form. Morphological and physiological characters are both changeable although not necessarily in a parallel way.

We shall now approach the question as to the possibility of filtering bacteria. The theory that bacteria are fungi imperfecti allows of two different possibilities: First, that a bud or a supposed endospore is so small that it is able to pass the filter: or second, that a piece of plasma containing nuclear substance can be sufficient to propagate the cell, as is the case with certain algæ. Attempts made by Almquist, by Löhnis and Smith, and by Jones, to filter the bacteria and obtain a new culture from the filtrate have not been followed with success. The only investigators that have succeeded in this undertaking are Hort and Mellon. The latter obtained from blood filtrate an invisible microörganism that developed into a polymorphous bacterium, and the former injected filtrates of spinal fluid from cases of meningitis into monkeys and obtained from the urine on agar among other forms the meningococcus (Weichselbaum) and diplococcus (Jaeger). By cultures Hort has been able to raise "giant meningococci," which watched on the warm stage show endosporulation and budding. In this connection, he writes:

> The meningococcus of Weichselbaum is not a bacterium. It is an ascospore derived from the "giant meningococcus" by a process of endosporulation. The filterable meningococcal virus probably represents a stage in the life-cycle of the ascomycetic organism described.

Hort considers the filterable form of importance in the spreading of meningitis, and a similar supposition concerning Pfeiffer's bacillus and influenza is held by Wade and Manalang, who found that this organism shows a fungus growth. Furthermore, Hort claims to have isolated from cases of typhus a very small bacillus that is polymorphous and pathogenic for monkeys. From these animals he later obtained the same forms, besides larger bacteria which he considers other developmental forms of the same organism. Considering the great possibility of contamination, it seems safe to regard all these cases with a certain amount of scepticism until they have been controlled on a larger scale. It seems to me not unlikely, however, that the typhus bacillus is a polymorphous bacterium. Pictures representing Ricketsia show a diplococcus-like microörganism whose two parts are connected with a thin filament. Such diplococcus-like forms are usually accompanied by rods and filaments. I should not be surprised if large portions of the group which Prowazek calls Chlamydozoa should turn out to be polymorphous bacteria.

In conclusion, a few words concerning what we call bacterial mutations. Most of the papers on this subject are of very limited value, inasmuch as they do not consider the normal variability of the bacteria. Where there is the possibility that a real mutation did occur, the question of contamination always raises doubt as to the interpretation of the observed facts. Almquist's bacillus antityphosus could possibly be explained as something similar to the phenomenon of d'Herelle, especially since Salimbeni has shown it to be probable that this phenomenon may be due to a parasitic organism living on B. Shiga-Kruse.

How much of this that I have presented is true, and how much of it is false, is at present impossible to say. But, at any rate, it seems that a great and promising field for investigation has been laid open. We must not forget that such groups as bacteria, protozoa and fungi are artificial divisions

made by us for our convenience. They have to be corrected as our knowledge develops.

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ERRATUM

Attention is called to an error on page 199 of the June BULLETIN in the article entitled "An Atypical Bacillus Paratyphosus B Infection" by Dr. Hugh J. Morgan. Table I in the left hand column of page 199 should be inserted between paragraphs 1 and 2 in the right hand column of page 199. See following arrangement:

At this time the patient's serum agglutinated a killed culture of B. paratyphosus B in dilutions of 1:50. There was no agglutination with B. paratyphosus A.

The low titer of our antiparatyphosus B serum against the unknown, together with the knowledge of the fact that organisms apparently falling into the paratyphosus B group are notoriously deceptive as to their identity, indicated the necessity of employing other methods for its identification. Castellani's absorption method was used. For purposes of simplification the organism under discussion will be called Bacillus "X" throughout the remainder of this report.

TABLE I

"A. T." Serum* v. B. paratyphosus B (N. Y.)	Before absorption	After absorption with Bacillus "X"
	Agglutination 1–2,500	Agglutination 1–2,500

"A. T." Serum v. Bacillus "X"	Before absorption	After absorption with B. paratyphosus B (N. Y.)
	Agglutination 1–1,000	No agglutination

*A polyvalent antityphoid serum containing agglutinins for B. typhosus, B. paratyphosus A and B. paratyphosus B.

Thus it was shown that Bacillus "X," while agglutinated by the specific paratyphosus B fraction of the "A. T." serum used, was unable to remove the paratyphosus B agglutinins from the serum.

THE CAPACITY FOR PHAGOCYTOSIS SHOWN BY POLYMORPHONUCLEAR LEUCOCYTES IN DEAD ANIMALS AND AFTER PRESERVATION IN SALT SOLUTION

By Howard B. Cross

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One of the disturbing surprises at autopsy is the occasional presence of leucocytes containing bacteria in transudates that have repeatedly been reported as sterile. This can in some instances, of course, be explained by assuming that bacterial invasion and phagocytosis have occurred during the agonal period. In many of the recorded cases, however, the clinical examinations and cultures have been made so recently before death that the remaining time is altogether inadequate to account for the extensive phagocytosis observed. Indeed, under some conditions the only admissible explanation for the presence of intracellular organisms is that the invasion and phagocytosis have taken place after the death of the patient.

Two opinions are generally entertained as to the significance of the occurrence of bacteria within phagocytes in the tissues and exudates of an infected animal after its death. The more common supposition which arises from this finding at autopsy is that the microorganisms were ingested by the phagocytes while the animal was yet alive. On this basis attempts are made to estimate the degree of resistance which the animal opposed against the infecting bacteria, and the phagocytosis is considered as an evidence in favor of the assumption that the microorganisms were etiologically important in the disease from which the animal suffered. While undoubtedly, in most instances, these assumptions are true, they should be weighed against the possibility that the phagocytosis observed at autopsy has occurred after the death of the animal.

The record in the literature of the continued activity of phagocytes independently of the functional coordination of the animal of which they were a part, is still fragmentary and incomplete. It, therefore, seems worth while to supplement this record with some disconnected observations on the viability of leucocytes made during the course of an experimental investigation of phagocytosis, which is being conducted in this laboratory.

There are a few recorded observations in the literature on the extension of the life of phagocytic cells after separation from the animal body. In a photographic study of the activity of the white cells of the blood Commandon[1] found that the leucocytes in blood preparations remained alive for 10 days if kept at a temperature of 10° C. When kept at 25° C., they continued alive for about 24 hours. However, when he attempted to preserve them at 38° C., their movements ceased and they died very soon. In 1915 Kobzarenko[2] noted that he was unable to find in the literature any indications on the capacity of leucocytes for independent existence. Accordingly he conducted a series of experiments and found that about 90 per cent of the leucocytes kept at incubator temperature were dead before the end of 48 hours. Approximately 50 per cent died during the first 24 hours. Weinberg and Sequin,[3] during an investigation of the inhibitory effect of hydatid liquid on phagocytosis, found that phagocytes immersed in this fluid were still capable of engulfing bacteria at the end of one hour. Hollande and Beauverie[4] demonstrated that leucocytes arising from a urethral exudate remained alive in the urine and exhibited phagocytic powers for as long as 10 hours, provided albumin were present. Wollmann[5] in an experiment of a somewhat different nature records that he kept leucocytes alive for 12 days within a collodion sac which was enclosed in the peritoneal cavity of a guinea-pig. Rowley[6] described a case of anemia in which the blood phagocytes, after being separated from the body for three months, were still capable of engulfing red cells.

In the above experiments wherever there has been any considerable extension of the life of the leucocytes after separation from the body, it has been accomplished by keeping them in the medium in which they occurred in the body. Then, after separation, the cell suspensions were kept at a constant temperature. Any alteration in the medium or marked change in the temperature seems invariably to have been accompanied with the most disastrous results.

The observations made in this paper were upon polymorphonuclear leucocytes and other cells subjected to the most varied circumstances. While the suspensions were sometimes kept in the refrigerator, no other precaution was observed to preserve the cells, and no special effort made to prolong their life, other than providing a trace of serum to prevent autolysis.

A sterile peritoneal leucocytosis was induced in each of four guinea-pigs by the injection of 5. c. c. of a suspension of aleuronat in physiological salt solution containing 3 per cent starch. Fifteen hours was selected as the time providing the maximum number of cells. These four animals were then subjected to the experiments described below.

Guinea-pig No. 1.—This animal was killed and left in the laboratory at room temperature for two hours. It was then injected intraperitoneally with a suspension of B. proteus. After one hour, samples of the exudate were withdrawn and examined. Many of the leucocytes had taken up the bacteria and were rapidly digesting them. Specimens of the exudate taken before the injection of organisms were culturally sterile and repeated smears did not reveal the presence of a single bacterium, intra- or extra-cellular. The microbes were taken up two to three hours after the death of the animal and in many cases were completely digested, as indicated by resolution into granules and loss of staining characteristics. Cells A and B in the accompanying plate illustrate the appearance of two representative phagocytes from this exudate.

Guinea-pig No. 2.—This guinea-pig was killed and after the removal of the abdominal viscera the animal was placed in the refrigerator and left there for seven days. At the conclusion of this time the peritoneum was washed out with salt solution, the resulting cells sedimented and a sample incubated with sensitized B. proteus for one-half hour. The most energetic phagocytosis ensued. Fifty

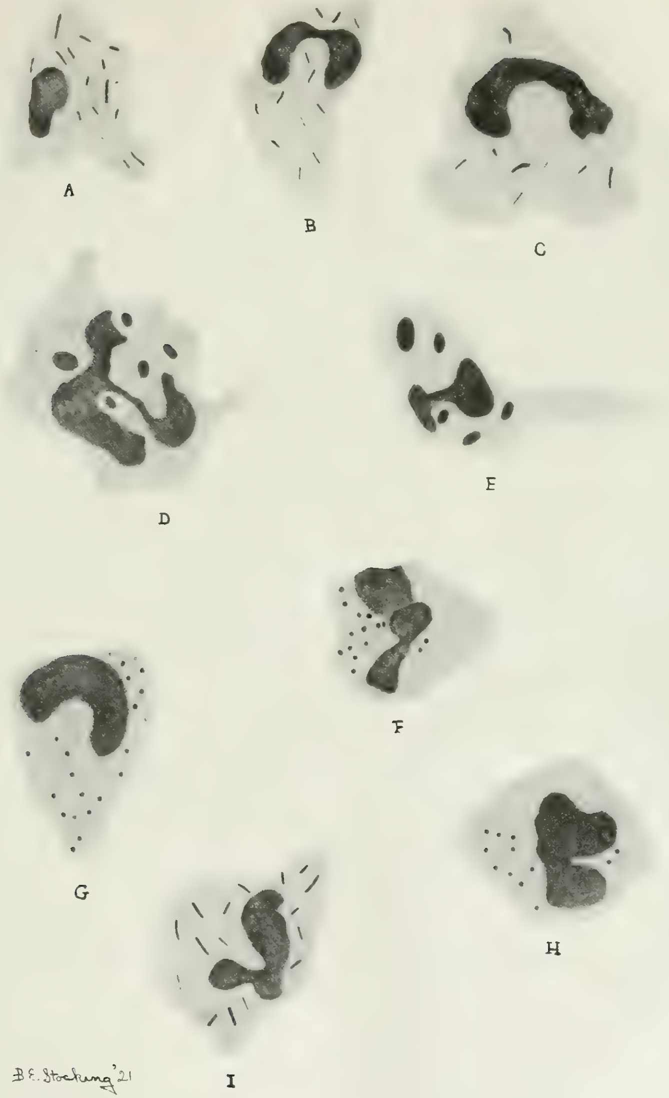

FIG. 1.—Camera lucida drawings (×1200) illustrating the appearance of phagocytic cells described in this paper. Leucocytes *A* and *B* represent an example of post-mortem phagocytosis. *C* is a polymorphonuclear leucocyte which, after 11 days in the dead body, was still actively motile and capable of phagocyting bacteria. *D* and *E*, leucocytes from the pleural cavity of a dog, remained active eight days after the death of the animal. The ingested yeast cells are surrounded by digestive vacuoles. Leucocytes *F*, *G*, and *H*, obtained from the peritoneal exudate of a guinea-pig, represent active phagocytosis after preservation in physiological salt solution for 18 days.

polymorphonuclear leucocytes counted contained more than 200 bacilli. Many of the cells were completely disintegrated, but others were apparently undisturbed and stained characteristically. The complete absence of bacteria in the original material was satisfactorily demonstrated.

Guinea-pig No. 3.—The peritoneal exudate was removed from this animal under sterile conditions and suspended in eight volumes of citrated salt solution (0.85% salt and 1.5% sodium citrate) and kept for six days. Four hours of each day the cells were kept at room temperature, four hours in the incubator, and the remaining time in a refrigerator with a temperature between 8° and 15°. Samples were removed each day and incubated with unsensitized bacteria. The results are given in the accompanying table.

Day	Number of cells	Serum	Number of Org.
1	50	None	125
2	50	None	135
3	50	None	121
4	50	None	160
5	50	None	120
6	50	None	00
	50	¼ vol.	500

The exudate was slightly hæmorrhagic and the presence of the extravagated serum probably accounts for the continued spontaneous phagocytosis. The experiment was discontinued on account of a contamination.

Guinea-pig No. 4.—The exudate from this animal was removed sterile and suspended in fifty volumes of citrated salt solution. The cells were kept for 18 days, part of the time at room temperature and the remaining time in the refrigerator. On incubation with sensitized staphylococci the first day the polymorphonuclear leucocytes ingested an average of seven bacteria each. A similar incubation on the third day resulted in a phagocytic index of five. On the eighteenth day only a few of the leucocytes were active and intact but phagocytosis was still evident and some cells took up as many as 12 cocci. Sketches F, G and H are made from three of these phagocytes.

A guinea-pig, which had died in the laboratory as a result of a pleural hæmorrhage after cardiac puncture, was placed in the refrigerator and left there for 11 days. At the conclusion of this time some of the fluid from the pleural cavity was withdrawn and on examination was found to be free of bacteria and to contain numbers of apparently normal polymorphonuclear leucocytes. A quantity of this material was incubated with sensitized B. coli. At the end of half an hour almost all the whole leucocytes in the smears contained from one to ten bacteria. The complete absence of organisms from the original material was conclusively established. An inspection of 100 cells did not reveal the presence of a single microbe after the incubation, whereas almost every polymorphonuclear leucocyte contained bacilli. Sketch C illustrates the appearance of a cell from these smears.

A dog died in the laboratory and at autopsy showed a pleural effusion containing a few unusually large polymorphonuclear cells. The animal was kept for three days at a temperature a few degrees above zero. Some of the fluid was then removed and kept in the laboratory for five days. The leucocytes had remained 72 hours in the body following death, and then, after being kept five additional days in a test-tube, were still actively motile and energetically ingested sensitized bacteria. Sketches D and E were made of phagocytes from this series.

A sample of citrated dog's blood was taken and left standing at room temperature for several hours. The sedimented cells were then placed in the refrigerator for four days. They were then removed and placed in the incubator for several hours. At the conclusion of this time a quantity of the leucocytes was incubated with sensitized bacteria, with a resulting energetic phagocytosis. Fifty cells counted contained 250 bacteria.

While there has been no opportunity or wish to subject human phagocytes to the rigid experimental conditions described above, the following data would seem to indicate that these cells too are capable of sustained independent existence and that they retain their functional capacities, and may reveal them under the most astounding circumstances.

The urine from a patient suffering with chronic urethritis was collected and left standing on the table for three hours. The sedimented cells were washed once, suspended in physiological salt solution and then placed in the ice-box for 24 hours. They were again placed on the table for one hour. The cells were next incubated for 15 minutes with sensitized bacteria. Enumeration revealed a phagocytic index of 17. (Sketch I.)

Polymorphonuclear leucocytes derived from this source have since been kept in this laboratory for as long as three days and when brought in contact with sensitized bacteria have revealed a phagocytic index only slightly lower than that obtained with freshly isolated cells. Indeed, it appears certain that the phagocytic index is influenced more often by the manipulation and medium than by the age of the cells.

The conditions recited above are, of course, exceptional. It is not always possible to duplicate these observations at will, and, indeed, it is often quite impossible except by accident. These results do, however, indicate that polymorphonuclear phagocytes possess to an extraordinary degree the capacity for independent existence, and when kept under appropriate conditions, removal from the body does not entrain, for a time at least, any demonstrable impairment of normal function.

DISCUSSION

It is possible that some may object to the instances of unusual phagocytosis presented in this paper, and insist that what has been interpreted as phagocytosis might better be explained by assuming that the cells and bacteria have been superimposed in smearing. Such hesitation, while perhaps thoughtful, rests upon an insecure foundation, for it is doubtful whether any phenomenon associated with phagocytic research is any more rare than "superimposition." Those investigating the phagocytosis of highly virulent bacteria often have to search through as many as a hundred leucocytes before finding a single intracellular organism, although the smears are crowded everywhere with extracellular bacteria. It is a common experience in smearing exudates in infections involving highly virulent bacteria to find the smear a veritable carpet of microorganisms broken only occasionally by empty

leucocytes. The appearance of vacuoles surrounding the bacteria, slight modification in staining characteristics, distribution of organisms within the cell, and progressive disintegration of the bacteria associated with loss of staining qualities, are unmistakable criteria of ingestion, which reduce to a minimum the possibility of error in interpretation. The cases of phagocytosis reported in this paper were so energetic and thoroughly characteristic from every point of view that it is impossible to regard the phenomenon as any other than the engulfment and digestion of bacteria by active, living cells.

SUMMARY

Polymorphonuclear leucocytes sometimes take up and digest bacteria within the body after death. In one instance these phagocytes remained alive within the body for 11 days after death and were still capable of energetic phagocytosis.

Leucocytes suspended in physiological salt solution, provided a trace of serum be present, may remain alive and manifest active phagocytosis for a considerable time.

Marked changes in the temperature of the phagocytes after separation from the body do not destroy and often do not even alter the capacity of the leucocytes for phagocytosis.

Polymorphonuclear leucocytes which had retained their functional capacities for three days within the dead body were removed and after being kept several days in a test-tube were still capable of energetically phagocyting bacteria.

Phagocytes from the blood and exudates seem to possess about equal tenacity of function.

Human leucocytes are capable of sustained independent existence.

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THE INGESTION OF MELANIN PIGMENT GRANULES BY TISSUE CULTURES

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Introduction

In the course of a study of the origin and development of melanin pigment in cultures of the pigmented layer of embryonic chick retina, it was noted that fibroblasts growing in the same culture ingested the free pigment granules (Smith, 1920). This observation suggested the idea of adding pigment granules to cultures of tissues from other parts of the embryo, with the view of ascertaining whether the power to ingest these granules was limited to certain cells or was a property common to the cells of all the tissues. Accordingly, cultures of connective tissue, skin, lung, liver, kidney, endoderm of intestine, peripheral nerves, striated muscle, smooth muscle, and the pigmented layer of the retina were obtained. Melanin pigment granules were secured from the eyes of the chick, the pig, the dog, and a new-born child. In addition to these, an amorphous precipitate of melanin, isolated from the urine of a man suffering from a melano-sarcoma, was employed in the experiments.

I wish to express my thanks to Mrs. Lewis and Prof. W. H. Lewis, in whose laboratory this study was conducted; also to Prof. D. Wright Wilson, of the Department of Physiological Chemistry of the Johns Hopkins Medical School, for assistance in isolating the pigment compound from the urine.

Technique

Small pieces of the tissue to be studied were explanted in Locke-Lewis solution. When these cultures were 24 to 48 hours old, a drop of fluid containing pigment granules from the eye of a child, pig, dog, or chick was added. The granules were obtained by teasing the retinal layer in a drop of sterile Locke-Lewis solution, the pigment imparting a light brown color to the medium. A little of the amorphous pigment, previously sterilized, was likewise added to a few drops of sterile Locke-Lewis solution and a small drop of the mixture placed on a number of cultures. Other cultures were explanted directly into media containing these different types of pigment granules.

The cultures were incubated at 39°C. and studied in a warm box. Some were fixed at various stages in Zenker's solution without acetic acid and stained with iron hæmatoxylin.

Observations

Pigment Granules.—The melanin pigment granules from the chick's retina were in the form of short rods and between dark brown and black in color. Their specific gravity was probably slightly higher than that of distilled water, as they settled out quite readily. They remained suspended, however, in Locke-Lewis solution. In the hanging drop of the cultures they exhibited two distinct varieties of movement. One of these apparently corresponded to what is known as Brownian movement; the granules furthermore actually progressed from place to place with remarkable rapidity. At first glance this second type of motion resembled that shown by an actively motile colon bacillus; on closer observation, however, it could be seen that frequently the granules whirled end-over-end as they travelled. The changes in their course were somewhat angular as compared with the more sinuous movements exhibited by the colon bacillus.

The amorphous pigment from the urine likewise remained suspended in Locke-Lewis solution and exhibited Brownian movement, but did not move from place to place. The granules from the eye of the pig and of the child behaved in exactly the same manner as did those of the chick. They were about the same size and form, except that their ends were slightly more pointed or cigar-shaped. The granules from the eye of

FIGS. 1, 2, 3.—Tissue culture cells fixed in Zenker's solution without acetic acid. Stained with iron hæmatoxylin.

FIG. 4. Cell from human liver fixed in Zenker's solution. Stained with hæmatoxylin and eosin.

All figures were drawn with camera lucida; No. 6 ocular and 2 mm. lens.

FIG. 1.—Normal pigmented cell from a 48-hour culture of the retina of a 5-day chick embryo. Each granule is an individual and discrete body of about the same size and shape. There are no vacuoles.

FIG. 2. -Fibroblast from a 48-hour growth of the subcutaneous tissue of a 7-day chick embryo. This cell has ingested melanin pigment granules from the retina of a 12-day chick embryo. Most of the granules are free in the cytoplasm; several are clumped together into pairs; and vacuoles are beginning to form about two of them.

FIG. 3.- Endothelial cell from a 96-hour growth of the liver of an 8-day chick embryo; the pigment within this cell is melanin pigment granules obtained from the retina of a pig. Two or three granules are free in the cytoplasm; other somewhat swollen granules are enclosed in vacuoles; several vacuoles are filled with fragmented granules; while others contain black homogeneous material. Near one side of the cell is a small irregular mass of finely granular debris.

FIG. 4.—A Kupffer cell from the sinusoids of the human liver. This cell has ingested malarial pigment. The masses of pigmented material are irregular in size and shape and a vacuole surrounds some of them. They resemble the ingested and partially destroyed pigment seen in Fig. 3.

the dog were found to be a little lighter in color than those of the chick and of four varieties: (1) small rods, similar in size and shape to those of the chick; (2) small round granules; (3) very large, oval or egg-shaped granules; (4) long spike-like granules over twice the length of those found in the chick's eye. The last three types were readily distinguishable from the normal pigment granules of the chick. The *dog* granules displayed essentially the same movements as the latter, but were probably a little heavier, as shown by their tendency to settle out of the drop.

The pigment appeared to be non-toxic, as no difference in the extent of growth of the tissue-culture was noted, even when the granules were so abundant as to color the medium.

In view of the fact that abundant fibroblasts were easily obtained from cultures of subcutaneous tissue, these cells were used to study the details of the ingestion process.

Fibroblasts.—Observations made immediately after the culture was treated with the Locke-Lewis solution containing pigment granules showed that a few of the granules had fallen on the tissue when the drop was applied, while the remainder were moving rapidly about in the fluid. As is usual in tissue-culture growths, the cells had migrated out on the under surface of the coverslip, so that during the procedure of adding a drop of pigment fluid some of the granules naturally settled on the cells, which, being rather sticky, held them. When the coverslip was reversed in returning it to the vaseline ring, these granules remained adherent to the under surface of the cell and were the first to enter it. Some of them were taken into the cytoplasm as early as 45 to 50 minutes after being introduced into the culture fluid. The free granules continued to move about in the fluid until they too became accidentally attached to the cells. When the granules were added directly to the culture medium at the time of explanting the tissues they remained suspended in the fluid until the cells grew out; then, as they moved about, some of them became attached to the surface of the cells and were subsequently taken into the cytoplasm.

In only a few instances did the cell make any active movement toward taking in the granule. This activity was shown by the slight bulging of the cytoplasm rather than the sending out of true pseudopodia. More often, however, the process would impress one as a gradual *sinking* of the granule into the cytoplasm. This, of course, is illusory, as the granule entered the cytoplasm from the under surface of the cell and could not *sink* upward. All the granules that adhered to a cell did not necessarily enter it. Some, for instance, after lying motionless under the cell for several minutes, or even a half hour, disengaged themselves and wandered off into the culture fluid, exhibiting the typical free-granule movement described above. Others, under apparently similar conditions, moved slowly up into the cytoplasm of the cell.

Although the pigment granules frequently entered the cytoplasm of cells containing vacuoles, they did not invade these bodies. Later vacuoles formed around the granules (Fig. 2), but the granules did not find their way into pre-existing vacuoles.

When free in the culture fluid the pigment granules exhibited the two kinds of movement described above: *i. e.*, Brownian and the progressive, end-over-end motion. After being taken into the cytoplasm of the cell, however, they showed the type of activity displayed by them when native in the pigmented cells of the retina (Fig. 1): *i. e.*, they started off with a spurt, proceeded a short distance, stopped suddenly and after a second or two of rest continued on. They sometimes moved back and forth in one region; again, they travelled entirely across the cell, passing either over or under the nucleus without apparently meeting any obstruction. When a granule first entered a cell it moved about freely, but usually within 3 to 24 hours a vacuole formed about it, due, perhaps, to the irritating effect of the granule on the cytoplasm. At first the vacuole was extremely small, appearing only as a slight halo around the granule (Fig. 2), the halo gradually enlarging into a definite vacuole (Figs. 2 and 3). For a time the granule continued its characteristic motion, carrying the vacuole about with it, but these movements became less and less extensive. As the vacuole increased in size, its contained fluid became much more refractive, so that the body was sharply marked off from the surrounding cytoplasm. When it became sufficiently large the granule within it exhibited Brownian motion. As the granule danced about in the vacuole, it began to swell (Fig. 3), tended to lose its rod shape, and appeared distinctly lighter in color. Whether this was due to an actual destruction of the coloring matter or merely a dilution of it, caused by the swelling of the stroma, could not be determined. Shortly after this the granule broke up into fragments. When this process of intracellular destruction had gone on for three or four days, there remained in the vacuole a small quantity of minute particles of debris.

Very often three or four granules appeared to have been agglutinated at one point in the cell, generally in the region of the centriole (Fig. 2) in which case one vacuole formed about them all. They all exhibited Brownian motion, gradually became swollen, lost their rod shape, and underwent the same process of disintegration and destruction as described above (Fig. 3).

This phenomenon could be studied to the best advantage with the aid of neutral red added to the cultures at the same time as the granules. When strong neutral red was used, the stroma of the melanin granules became stained (Smith, 1920) and remained red even when the granules were attached to the cytoplasm, although the cytoplasm in contact with them displayed no color. In most instances a weak solution of neutral red was used, and in these the free pigment granules remained unstained; even when they became attached to the cell, neither they nor the cytoplasm showed any evidence of staining. When vacuoles or neutral-red granules were already present in the cell, these at once took the stain, while the other components of the cell remained colorless. As stated above, when a pigment granule penetrated a cell it did not enter one of these pre-formed vacuoles, but moved back and forth in the colorless cytoplasm. Very soon, however (10 to 30 minutes), a narrow pink zone could be seen extending around the periphery of the granule. This was the intial stage in the

formation of the vacuole and could not be made out without the aid of the neutral red. The pink band grew gradually wider and deeper in color until there resulted a relatively large vacuole stained bright red. The stroma of the granule within the vacuole now became red also and retained this color throughout the entire process of dissolution. Even more striking was the fact that the debris resulting from the destruction of the granule retained the red color, even after fixation in Zenker's solution and staining with iron hæmatoxylin.

Although in some cases as many as twenty pigment granules were seen in a single fibroblast, it was not usual for these cells to contain more than about eight. The penetration by one granule semed to predispose the cell to further invasion; or it may have been that the cytoplasm of that particular cell was in a state to admit the granule more easily. For instance, one cell might have six or eight granules, while another cell growing beside it had none. There was nothing in the appearance of the cells in question to explain this difference in reaction. The entrance of granules into a cell evidently had some deleterious effect upon it, as such cells were observed to form degeneration vacuoles more frequently than cells in the same culture that had not taken in any granules.

No difference in behavior was noted between fibroblasts grown from the various tissues, whether subcutaneous, kidney, or lung tissue.

Clasmatocytes.—A certain number of clasmatocytes were nearly always present in these cultures, regardless of the kind of tissue from which the explant was made. These were large migrating cells whose cytoplasm contained numerous granules and fat globules. There were usually several large vacuoles present in these cells, even in healthy cultures in which no fibroblasts or other cells contained vacuoles. The clasmatocytes were characterized by numerous finger-like processes reaching out in all directions. They curved and twisted down into the hanging drop more often than they extended along the coverglass. While these pseudopodia displayed great activity, they never seemed to flow out like those of leucocytes or amœbæ.

The pigment granules appeared in clasmatocytes in large numbers and much more rapidly than in the fibroblasts. The phenomenon began almost immediately (10 to 20 minutes), and by the end of two hours many of these cells appeared quite black with granules, while the neighboring fibroblasts contained only a few. Individual clasmatocytes were observed to take in 30 or 40 granules. Some of the cells into which a large number of granules entered died shortly afterward. In preparations stained with neutral red this was indicated by the fading out of the stain from the vacuoles.

The granules did not move about from place to place in the clasmatocytes as freely as in the fibroblasts, but the vacuoles appeared to form with even greater rapidity. After a few hours a number of irregular masses of melanin were present in the cells. A vacuole was not readily distinguishable about some of these masses and in a few cases seemed to be entirely lacking. The process of decomposition of the melanin granules was essentially the same as in the fibroblasts, except that the destruction of the granules was not so complete.

Bloods-Cells.—Many of the cultures contained both red and white blood-cells. No pigment granules were observed within the red blood-cells, nor were any seen to be taken in by them. Generally, the white blood-cells were slightly more active than the fibroblasts but not nearly so active as the clasmatocytes. After the granules entered, vacuoles formed about them and the process of destruction proceeded as described above.

Epidermal Cells.—In view of the fact that in animals the epidermis is so often pigmented, cultures from that tissue of the chick were studied with considerable interest. About the same number of melanin granules were found in the epidermal cells as in the fibroblasts of the same culture. The vacuoles were more tardy in forming around the granules, but the granules were broken up eventually into finer particles than in the fibroblasts. In all other respects the processes were identical.

Alveolar Cells of the Lung.—Excellent growths from the cells forming the alveoli of the embryonic lung were obtained. The behavior of these cells toward the pigment granules was practically the same as that of the fibroblasts.

Liver Cells.—As a rule, the liver cells did not take in the granules in quite such large numbers or so rapidly as the fibroblasts. In these cultures certain other cells which resembled to some extent clasmatocytes but which were possibly primitive Kupffer cells, did, however, ingest the granules in large numbers and with considerable rapidity. In the liver cells, also, the granules were taken directly into the cytoplasm and later vacuoles formed about them. Bile was present in these cells but was not observed in the vacuoles that formed about the pigment granules. So far as could be made out, the granules were more nearly destroyed in the liver cells than in any others.

Endoderm of the Intestine.—In cultures of the intestine the fibroblasts grew out in strands, while the endoderm grew only in sheets. No essential difference was noted between the endodermal cells and the fibroblasts in their reaction toward the pigment granules. The granules were taken in, vacuoles formed about them, and they became broken up into irregular clumps of debris.

Kidney.—Cells of the kidney tubule grew out in the form of a thin membrane much like that observed in the liver cells. The pigment granules were taken into these cells and became broken up into fine particles similar to those found in the skin cells.

Striated Muscle.—In explants of striated muscle the cells grew out as long, spike-like strands containing many nuclei. No pigment granules were found within these muscle-fibers.

Smooth Muscle and Epithelium from the Amnion.—Two types of cells were obtained from explants of the amnion: epithelial cells which grew out in the form of a membrane, and smooth-muscle cells which grew out individually like the fibroblasts. The epithelial cells ingested granules in large numbers, being exceeded in this respect only by the clasmatocytes. Vacuoles did not form about the granules in these cells so rapidly or so extensively as in cells of other types. Some of the granules remained free in the cytoplasm for 24 hours or longer. The granules were eventually broken up into

fine particles, as noted in the ectodermal cells and in the cells of the kidney tubules. They were not found in clumps within these cells.

The smooth-muscle cells did not take in the granules when they first grew out nor as long as they were exhibiting rhythmical contraction; but when the cells had become spread out like fibroblasts, granules entered, vacuoles formed about them, and they disintegrated, just as in true fibroblasts.

Nerve Cells.—Sympathetic nerve cells were seen in cultures from various tissues. These were always free from pigment granules.

Pigment Cells.—Since pigment granules were taken in by many kinds of cells, it was deemed of interest to see if the pigment cells of the retina were themselves capable of ingesting foreign pigment granules. Granules from the dog's eye were added to a number of membranes of pigment-bearing cells from the retina of the chick, for the reason that these could be easily distinguished from the native pigment granules. They showed the same peculiar movement in the culture fluid as did the granules of the chick, but most of them sank to the bottom of the culture drop. In order that the pigment granules might be brought in contact with the growing cells, the cultures were incubated upside down for 12 hours. At the end of that time the majority of the cells had taken in large numbers of the foreign pigment granules. These were usually collected within one large vacuole and there was some evidence of their destruction.

Pigment Cells of the Fish.—While studying cultures from the embryo of the fish (Fundulus) Mrs. Lewis noticed that many of them contained pigment cells. A few of these cultures she kindly fixed for me. It could be readily seen that here we were dealing with two distinct types of cells containing pigment. The first was a rather large cell with numerous branched processes. This was the true chromatophore which is so common in reptiles, amphibians, and fish. These cells were snugly filled even to the ends of their processes with more or less rounded granules, each of which could be made out as a separate unit. In some of these cells the granules were black, in others yellow, reddish, or light brown; but the striking fact was that no single cells had granules of more than one color. The second type of cell containing pigment granules was the mesodermal cell, which frequently contained granules of more than one color. These were clumped together in vacuoles, exactly as in the cells of the chick embryo described herein. This evidence all points to the fact that these cells had ingested and not formed the granules.

Discussion

From my studies on the behavior of the cells of tissue cultures toward the pigment granules, when the two structures are brought together under experimental conditions, it seems to me that the melanin granules have special advantages over other materials used in the study of ingestion. Their dark color makes them easy to follow, and one can tell from their behavior exactly what part of the process is going on; *i. e.*, when the granule is free in the culture fluid, it exhibits both Brownian motion and actual progression; when attached to the cell, it is motionless; when free in the cytoplasm, it displays the jerky motion characteristic of the native pigment granules; and finally, when surrounded by a vacuole, it again undergoes Brownian motion.

The method by which the melanin granules make their entrance into a cell invites speculation as to the factors involved in the phenomenon. Certainly it appears to be very different from the manner in which an amœba is supposed to engulf its food. The cells do not send out pseudopodia and surround the granules;[1] nevertheless, from all appearances, it seems quite certain that the process depends more upon the cytoplasm than upon the activity of the granules themselves. From my observations there would seem to be at least two stages in this process. In the first the granule becomes attached to the side or under surface of the cell: in the second stage it enters the cell and moves about freely within the cytoplasm.

The question arises: How does the granule get from the external surface to the interior of the cell? It seems most probable that the phenomenon of capillary attraction is the basic principle, but in the application of this principle we have two possibilities to consider: In the first place, is the process simply a pulling in of the granule once it is in intimate contact with the cytoplasm? Or, must there be some local modification of the cellular wall before the factor of capillary attraction can come into play? The varying lengths of time required for the completion of the process in different types of cells point to the latter as the more probable solution.

A large variety of cells have been shown to possess the power of ingesting foreign material. A long series of dyes (Evans and Schulemann, 1914, Clark and Clark, 1918), carbon granules (MacCallum, 1903), fine particles of certain metals (Plato, 1900), melanin pigment granules (Matsumoto, 1918), and also bacteria (Metchnikoff, 1905, Plato, 1900; Evans, 1915; Jones and Rous, 1917; Lewis, 1920) have been used in the study of this phenomenon. While certain of these observers occasionally mentioned vacuoles in connection with the ingested foreign body, it had not been definitely stated that vacuole formation always accompanied such ingestion until 1919, when Shipley stated (page 293) that "all sorts of material which the cell allows to enter, or takes into, its cytoplasm, eventually finds its way into some of these vacuoles of segregation." On page 297 he asserted: "When large foreign bodies are ingested, they are enclosed in a fresh vacuole formed during their engulfing."

However, in the case of the melanin granule and also the avian tubercle bacillus,[2] it was clearly evident that the foreign

[1] Dr. Mary Hogue, of the School of Hygiene, Johns Hopkins Medical School, noted that amœbæ in tissue cultures ingested melanin pigment granules obtained from the eye of an embryo chick. This particular species of amœba did not "ingulf" the pigment granules, neither were the granules taken into food vacuoles, nor were vacuoles formed about them coincident with their entrance into the cell.

[2] Certain experiments were undertaken in order to determine whether cells ingest and destroy bacteria in the same manner in which they take in and destroy pigment granules. Suspensions of avian tubercle bacilli, obtained through the kindness of Dr. H. S.

body was not taken into a preformed vacuole, nor did a vacuole form about it coincident with its entrance into the cell, as claimed by Shipley; it was only after the granule had been in the cytoplasm for some time that a vacuole was formed about it by the cytoplasm. This vacuole may have contained digestive enzymes, oxidizing agents, or other substances which acted upon the melanin in such a way as to cause it to break up into particles. It was evident from the neutral red reaction that the vacuole was slightly alkaline. The protein composing the stroma of the melanin granules is one which does not easily undergo decomposition, as has been shown by Abel and Davis (1896), who found that these granules were so resistant outside the body that they were not destroyed by concentrated HCl, and were broken up only after prolonged treatment in the water-bath in dilute (5-10 per cent) KOH.

In this and previous studies it was observed that the granules within any particular true pigment-producing cell were always individual and discrete bodies of about the same size, color and shape (Fig. 1). Individuality and discreteness were common properties of the granules in all true pigment-producing cells, but color, size and shape varied according to the origin of the cell. The granules were never found clumped together or in vacuoles in these cells. On the other hand, granules or other particles of pigmented material, when ingested, were usually found clumped into masses of irregular size and shape, enclosed in vacuoles, or broken up into debris (Figs. 3 and 4).

Combining these two observations, it can be seen that here is a possible method of determining whether the pigment granules within a certain cell were produced by that cell or were merely taken in by it from the surrounding medium. Several hundred sections of tissue, both normal and pathological, including a large number of pigmented tumors, have been examined, and the indications are that this criterion will enable us to settle the old question of pigment-producing (Fig. 1) versus pigment-carrying cells (Figs. 2, 3, and 4). In a given pathological condition (Fig. 4), where pigment is present, one can tell whether the cells are producing or ingesting it. In the case of tumors one can distinguish between the true pigmented tumors (those arising from pigment-bearing cells) and those in which pigment occurs as a result of the cells ingesting melanin pigment, blood pigment, or both.

SUMMARY

(1) Melanin pigment granules from the retina of the chick, the pig, the dog, and man (child) showed a variety of movements which enabled one to identify the position of the granule, *i. e.*, to determine whether it was free in the culture fluid, adhering to the cell, within the cytoplasm, or within a vacuole.

(2) Flakes of an amorphous precipitate of impure melanin pigment, isolated from the urine of a man suffering with extensive metastases from a melano-sarcoma, showed Brownian motion but did not progress from place to place.

(3) Fibroblasts, clasmatocytes, endothelial cells, white-blood cells, epidermal cells, alveolar cells of the lung, liver cells, kidney tubule cells, endodermal cells of the intestine, pigment cells of the retina, flattened out smooth-muscle cells, and epithelial cells of the amnion of the chick ingested melanin pigment granules.

(4) Red-blood cells, striated-muscle cells, rhythmically contracting smooth-muscle cells, and certain peripheral nerve cells, occasionally found in the cultures, did not ingest the granules.

(5) The pigment granules were taken directly into the cytoplasm of the cell and not into preformed vacuoles; as the granule moved back and forth in the cell, however, a vacuole formed about it. Within the vacuole the granule exhibited Brownian motion. Gradually, it was reduced to tiny particles of debris. Frequently a number of granules became clumped together, one vacuole forming about them all. The clumping of the granules together into large masses did not take place so extensively in the ectodermal or epithelial cells, but in these each granule was broken up into very fine particles.

(6) Mesodermal cells of the fish embryo (Fundulus) ingested pigment granules liberated from the chromatophores of the fish.

(7) Native granules in the true pigment-producing cells are always individual and discrete bodies of about the same size and shape. They are never found clumped together in vacuoles or broken up into fragments. It can thus be determined by the appearance of the granules whether they have been produced within the cell in which they are seen or have been ingested.

Willis, of the Kenneth Dows Tuberculosis Research Laboratory, Johns Hopkins Hospital, were placed upon tissue cultures. It was soon evident that the manner in which the bacilli entered the cell and the vacuoles formed about them was much the same as that exhibited by the melanin granules. The details of these observations will be described in a separate publication.

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CHRONIC MENINGOCOCCUS SEPTICÆMIA

A REPORT OF TWO CASES

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During the spring and summer of 1920 there came under observation at The Johns Hopkins Hospital two examples of an unusual form of meningococcus infection, namely, chronic meningococcæmia. In both instances the organism was isolated in pure culture from the blood stream during the course of a long septic disease. The infrequency of the condition, the difficulties met with in its diagnosis, and the gratifying therapeutic results obtained by the use of specific sera, render it allowable to place these cases on record.

Meningococcæmia

Although the first authentic case of meningococcus septicæmia was reported by Gwynn in 1898, as early as 1881 Gaucher had isolated a micrococcus from the blood of a patient who later died of meningitis, and it is fairly certain that he was dealing with the same organism which Weichselbaum, six years later, called *Diplococcus intracellularis meningitidis*. Next after Gwynn's report came that of Saloman in 1902, and was followed by those of Warfield and Walker, Claude and Bloch, Rist and Paris, Martini and Rhode, Andrews, Simon, Liebermeister, Bovaird and others. French observers, more particularly, have called attention to the various clinical manifestations of this condition, most prominent among them being Netter, Claude and Bloch, Rist and Paris, Monziols and Loiseleur, Chevrel and Bourdenier, Pissavy, Richet the younger and Pignot, Portret, P.-L. Marie, Sainton and Maille, Lancelin and others.

It has been known, then, for many years that a meningococcus infection may give rise to a general septicæmia. Nevertheless, the overwhelming predominance of meningitic manifestations in instances of meningococcus infection has obscured this fact rather naturally, and one is apt to associate the organism only with cerebrospinal fever. But that it may cause lesions elsewhere, independent of, or associated with, a meningitis, has long been known.

Josia (cited by Portret) and Gwynn (in 1898) were the first to isolate the meningococcus from suppurating joints in cases of cerebrospinal meningitis. Canult described pericardial lesions, and Warfield and Walker, and Claude and Bloch observed endocardial lesions, caused by the same organism, in 1900 and 1903, respectively. Jacobitz, in 1905, reported a case of meningococcus pneumonia, followed in a few days by a meningococcus meningitis. Achard and Flandrin (cited by Portret) also noted this unusual localization. Iridocyclitis caused by the meningococcus has been described (Bovaird), as well as cerebral abscess (Manziols and Loiseleur).

When one considers that the lesions enumerated above occur in cases associated with, or independent of, cerebrospinal meningitis, he is forced to the assumption that invasion of the blood stream is not an uncommon occurrence in meningococcus

infections, and that these lesions are metastatic. Moreover, the observation of Herrick in this country, that fully 50 per cent of his cases of cerebrospinal meningitis clinically were preceded by a meningococcæmia, lends weight to this opinion which had been strongly insisted upon by Elser and Huntoon in 1909. Of this type of meningococcus infection (*i. e.*, meningococcus septicæmia) our present cases afford examples, and in order that their relative place among the meningococcus bacteræmias may be defined, a clinical classification of meningococcæmia, based on reported cases, will be given:

A. *Fulminating Meningococcus Septicæmia.*—This form represents an overwhelming infection, characterized by an abrupt onset with chill, headache, prostration, a purpuric skin eruption, a high or subnormal temperature, with collapse and death usually within 24 hours. The diagnosis is made only at autopsy (cultures) or by blood cultures taken before death from a vein or from a purpuric spot. The fulminating cases of so-called cerebrospinal meningitis undoubtedly fall into this group, many showing at section no central nervous system lesions, and others only slight evidence of meningeal localization of the infection. A number of cases, representing this form of sepsis, in which the bacteræmia has been proven by ante-mortem blood cultures, have been reported (Andrews, Anderson, Pybus, Herrick, Worster-Draught and Kennedy, Sophian, and others).

B. *Acute Meningococcus Septicæmia.*—The majority of cases of acute cerebrospinal meningitis probably fall into this group. In such instances the organisms more or less rapidly localize in the meninges, and the blood becomes sterile. It would seem highly probable that the comparatively low percentage of positive blood cultures in cases of acute cerebrospinal meningitis is dependent entirely upon the rapidity of meningeal localization, since, as a rule, after the meningitis has definitely developed, the bacteræmia disappears. Certain it is that there are cases of meningitis which are preceded by symptoms of a severe general infection of several days' duration, and in which a meningococcæmia is proved by blood culture. Many instances of this sort have been cited in the literature (Gwynn, Netter, Pissavy, Richet the younger and Pignot; Lemierre, Portret, Sophian, Worster-Draught and Kennedy, and others) and their occurrence is not uncommon in extensive epidemics of the disease.

C. *Chronic Meningococcus Septicæmia.*—It is this infrequent form of meningococcæmia to which we wish particularly to direct attention, inasmuch as our two cases fall into this group. The case histories will be followed by a discussion of the diagnostic and therapeutic problems which arise in connection with the group.

Case I.—Name: G. W. Age: 33. Sex: Male. Race: White. Occupation: Shipyard employee. Admitted June 22, 1920. Discharged August 16, 1920.

Summary.—Onset sudden, following extraction of a tooth; chills, fever, lassitude; recurring crops of erythematous nodules; joint pains; headache; weakness and loss of weight. Symptoms persisted for 56 days without change. Blood culture positive for meningococci on the 49th day, seven days before development of signs of meningitis. Cerebrospinal meningitis. Antimeningococcus serum intravenously (110 c.c.) and intraspinously (225 c.c.). Serum sickness. Recovery.

Complaint.—"Aching and numbness in legs; lumps on legs."

Family History.—Unessential.

Past History.—His general health had been excellent. He had measles and mumps in childhood, but has had no other illnesses. His habits were exemplary.

Present Illness.—On May 22 (one month before admission) a tooth was extracted, with great difficulty, under local anæsthesia. The tooth was broken several times during the procedure and a great deal of cocaine was used. That same evening he felt hot, and "had chilly sensations up and down the spine." On the following day he felt weak, his arms and legs ached, and were stiff. Hard, red nodules, varying in size from approximately 1 to 3 mm., appeared in the skin of the extremities. These nodules were somewhat tender. After a few days they disappeared.

After May 22 the patient had "chilly sensations," lasting from five to ten minutes every night and accompanied by fever. There were no sweats. The stiffness and soreness of the muscles of the extremities continued, and crops of the subcutaneous nodules appeared at intervals of from three to five days. They would gradually disappear, to be replaced by a fresh crop of lesions. During the first week of his illness he continued to work, but increasing weakness and malaise, together with the symptoms mentioned above, forced him to stop, and he had been confined to his bed since.

From the onset the patient had been troubled with anorexia and constipation. He stated that he had lost ten pounds in weight during the month previous to admission.

Physical Examination.—The temperature was 99.2° F., the pulse 80, and the respirations were 20 per minute. The blood pressure was 85 systolic and 40 diastolic. There was evidence of some recent loss of weight, and the skin and mucous membranes showed slight pallor. Scattered mainly over the extensor surfaces of the forearms and legs, and to some extent over the flexor surfaces, were numerous red and bluish-red macular and papular skin lesions, varying in diameter from 1 mm. to 2 cm. The distribution was somewhat striking, in that the lesions were for the most part confined to the extremities, the lower extremities showing more than the upper ones. Examples of all stages of development of the lesions were present—small, slightly raised, circumscribed, erythematous spots; larger, definitely elevated, red papules which were tender; still larger, dark bluish nodules 0.5 to 2 cm. in diameter, which were moderately tender. Scattered over the skin were circumscribed, very slightly pigmented areas, which were interpreted as the sites of old lesions. There was no general glandular enlargement. The examination of the head and neck showed only a small, granulating slough at the site of the extracted tooth, and evidences of chronically infected tonsils. The eye grounds were normal. Examination of the heart and lungs was negative. The pulse was soft and a little rapid. The abdomen was entirely negative; the spleen was not enlarged to percussion. Examination of the genitalia, joints and reflexes revealed no abnormalities. The rectal examination (digital) was negative.

Laboratory Examinations.—Blood: Hæmoglobin (Sahli), 80 per cent. Leucocyte count, 12,600. Wassermann, negative. Blood culture (June 23), no pathogenic organisms. Blood culture (June 26), sterile. Urine: several examinations were entirely negative, with the exception of a faint trace of albumin on one occasion.

Course in Hospital.—During the first four days of his stay in the hospital the patient ran a high intermittent fever, and there were chills and "flushes." No sweats occurred. The eruption present on admission almost entirely faded away during this time. A new nodule was noted on the left forearm, but this disappeared in forty-eight hours. The patient complained bitterly of pains in the joints and of headache. His general appearance strongly suggested sepsis.

On June 27 the patient was without fever for over 24 hours, and felt considerably better. On the following day he had a rather sharp chill, and the temperature rose abruptly to 104.5° F. Fresh erythema-

tous nodules appeared at this time, and continued to do so every few days.

During the first two weeks of July his condition remained essentially unchanged. He continued to run an intermittent, septic type of fever, associated with either definite chills or chilly sensations. His spleen became palpable during this time. He gradually became weaker and there was some loss of weight. He slept a great deal of the time, and when awake would complain of headache and pains in his joints. No evidences of meningeal involvement were present throughout this period. On July 16 his temperature rose to 105.4° F., the pulse to 110 and the respirations to 26 per minute. He seemed definitely worse, and was much more irritable and restless. On July 17 vomiting occurred and for the first time there was some rigidity of the neck. Kernig's sign was present and there was moderate exaggeration of the deep reflexes. A lumbar puncture revealed a slightly hazy cerebrospinal fluid, under normal pressure, containing 9480 leucocytes per cubic millimeter. Of these cells 98 per cent were of the polymorphonuclear type. A few Gram-negative intracellular diplococci were seen in the smear. A blood culture taken July 10 was reported to contain meningococci of the "normal" type. There were about six colonies per cubic centimeter of blood. Forty cubic centimeters of antimeningococcus serum were given intraspinously, and 40 c. c. intravenously. Cultures of the cerebrospinal fluid taken on this date (July 17) showed the meningococcus, "normal" type; a blood culture was sterile.

July 18.—Fifty cubic centimeters of antimeningococcus serum were administered intraspinously and 70 c. c. intravenously. The patient seemed somewhat improved; he was less drowsy and complained less of headache. The cerebrospinal fluid contained no organisms demonstrable by smear or culture.

July 19.—The patient was still febrile (see chart). Antimeningococcus serum (45 c. c.) was given intraspinously. No organisms were obtained from cultures of the cerebrospinal fluid.

July 20.—Antimeningococcus serum (45 c. c.) intraspinously.

July 21.—Antimeningococcus serum (45 c. c.) intraspinously. Cultures on the 20th and 21st revealed a sterile cerebrospinal fluid, although it was still somewhat turbid. The patient showed considerable improvement. A blood culture taken on the 20th was sterile. Cultures from the nose and throat and from the site of the extracted tooth were negative for meningococci.

July 22.—The patient developed the typical urticarial rash of serum sickness. A low intermittent fever persisted. The eyegrounds showed a slight hyperemia of the discs and fulness of the veins. Some retraction of the neck, and Kernig's sign, were present. These symptoms persisted for a few days and then gradually disappeared.

July 24.—The cerebrospinal fluid was clear.

July 30.—A blood culture was sterile. Lumbar puncture resulted in the recovery of clear cerebrospinal fluid, under normal pressure, containing 14 cells per cubic millimeter; it was sterile on culture.

With the disappearance of the serum sickness the patient's general condition rapidly improved. There were no recurrences of the headaches, joint pains or skin manifestations, and he was discharged August 16 entirely well, his convalescence to be completed at his home.

Case II.—Name: V. F. Age: 28. Sex: male. Race: white. Occupation: lawyer. Admitted, July 8, 1920. Discharged, August 25, 1920. Result: well.

Summary.—Weakness and lassitude; sore throat; slight fever; headache; general muscular pains. Physical examination negative except for chronic tonsillitis and slight oral sepsis. Intermittent fever. Slight leukocytosis with polymorphonuclear percentage increase. Development of tender subcutaneous nodules; tonsillectomy; cerebrospinal fluid contained 36 cells per c. mm. and gave a positive globulin test. On the 39th day a blood culture (6th) was positive for a meningococcus (paranormal type); specific serum treatment (intravenously—75 c. c., intraspinously—155 c. c.); serum sickness; recovery.

Complaint.—"Headaches; aches all over; fever."

Past History.—Measles and mumps in childhood and an uncomplicated case of scarlet fever at the age of eight.

For several years previous to his admission to the hospital the patient had been subject to severe daily occipital headaches. During the last two years these had gradually decreased in severity.

About ten years before admission he had a transient attack of jaundice, without symptoms suggestive of cholelithiasis or serious gall-bladder or bile-duct disease.

On admission the patient stated that his general health had been rather poor for several years; that he had to force himself to his work and tired easily, and that altogether he "did not feel up to par." However, his appetite had remained good, and he had noted no loss of weight. He smoked cigarettes in moderation. No excesses of any kind were indulged in.

Present Illness.—For three months previous to his admission the feeling of lassitude and inefficiency noted in the past history had increased. Two weeks before admission to the hospital he noted that he was losing weight and that the slightest exertion tired him. His throat was somewhat sore, and he thought that he had some fever. He felt worse in the evening, when headache became a prominent symptom. He also had slight general muscular soreness.

He entered the hospital more on account of his general run-down condition than for the relief of any specific symptoms.

Physical Examination.—The temperature was 99° F., the pulse 72 and the respirations 20 per minute. Blood pressure—105 systolic, 65 diastolic. The general nutrition was rather poor. The skin was slightly sallow, but otherwise entirely negative. The glands at the angles of the jaw were palpable; there was no general glandular enlargement. Examinations of the eyes and ears were negative. The teeth showed some slight evidence of pyorrhœa. The tonsils were large and several of the crypts contained plugs. There was a slight diffuse enlargement of the thyroid gland, but no signs of hyperthyroidism were elicited. Examination of the lungs revealed possibly a slight impairment of the percussion note over both apices, but no changes in the breath sounds were present, and there were no râles. The heart was normal in size and position and the sounds were clear. The peripheral vessels were soft. Examination of the abdomen was entirely negative. The genitalia and extremities were normal. No clubbing of the fingers or toes was present. The superficial and deep reflexes were present and normal.

Laboratory Examination.—Blood: R. B. C., 4,512,000. Hæmoglobin (Sahli), 85 per cent. Color index, 0.9. W. B. C., 10,600. Aside from slight pallor of the red cells and the leukocytosis the stained film revealed nothing abnormal.

Differential count:

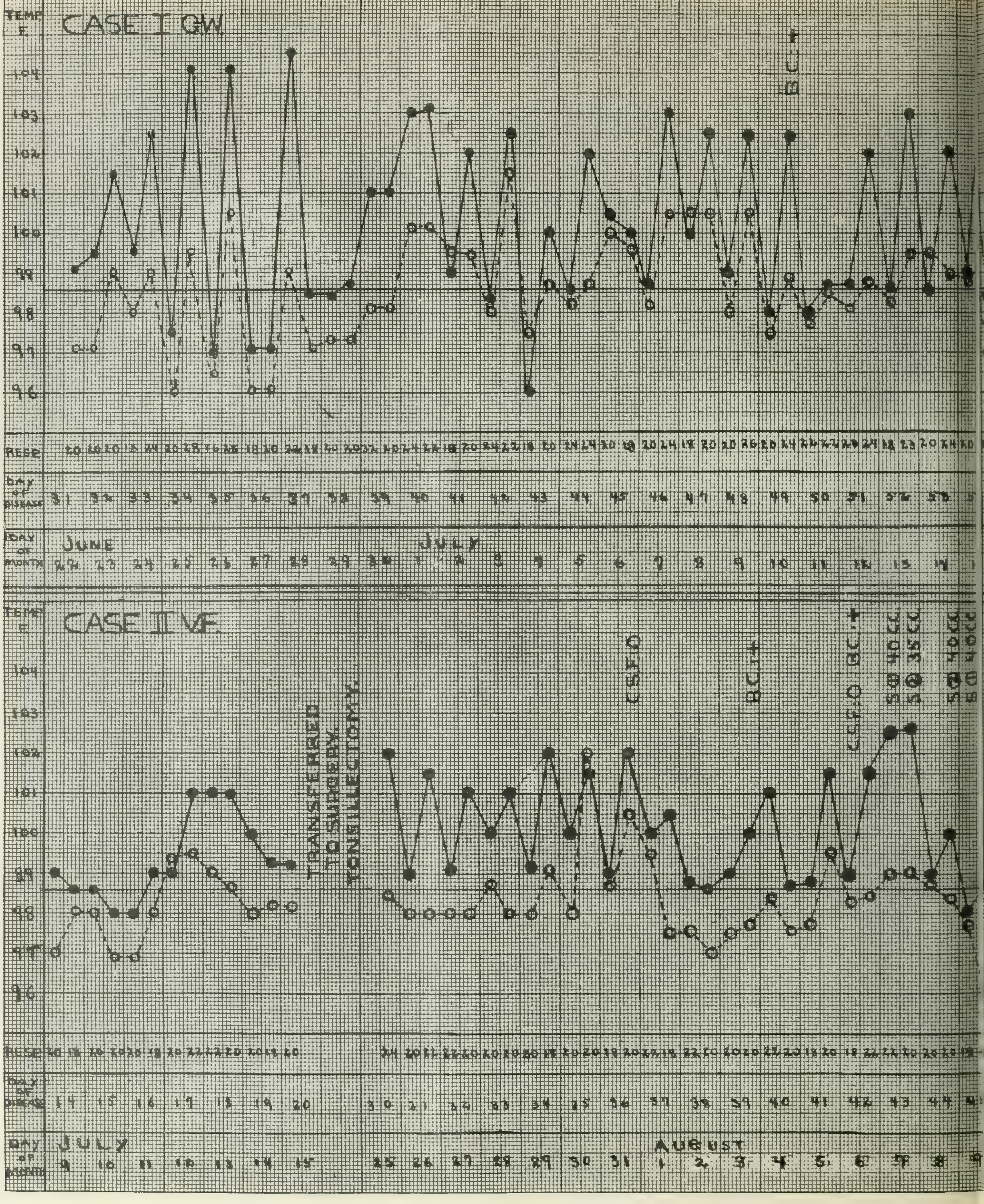
	Per cent
Pm. N.......	73.5
Pm. E.......	0.5
Pm. B.......	0.0
Large mononuclear cells......	19.5
Small mononuclear cells......	2.0
Transitional cells......	4.5
	100 (200 cells counted.)

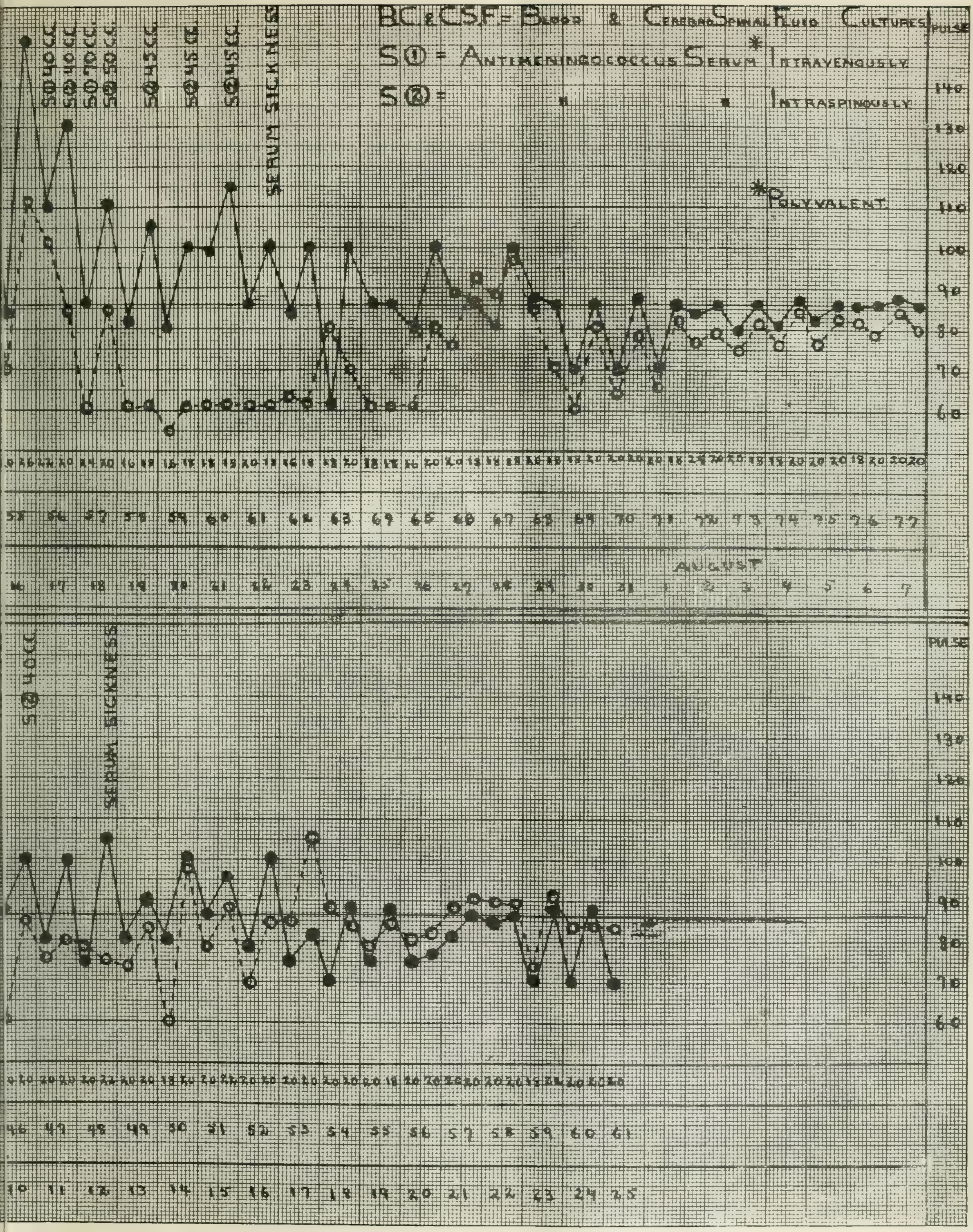
Urine: S. G., 1020; acid; albumin—trace; an occasional hyline cast. Tests for bile and blood were negative. The day after admission the trace of albumin disappeared, and no casts were found on microscopic examination.

Wassermann reaction (blood), negative.

Blood cultures made on the day of admission, and repeated two days later, remained sterile.

An x-ray plate of the chest revealed moderate fibrosis of both lung apices—probably an old tuberculous process. An examination of


CASE I G.W.
B.C.+
TEMP. F.
104
103
102
101
100
99
98
97
96
RESP.
DAY OF DISEASE
DAY OF MONTH
JUNE
JULY
CASE II V.F.
TRANSFERRED TO SURGERY. TONSILLECTOMY.
C.S.F.O
B.C.+
C.S.F.O B.C.+
S.O 40 CC.
S.O 35 CC.
S.O 40 CC.
S.O 40 CC.
AUGUST


BC & CSF = Blood & CerebroSpinal Fluid Cultures
S① = Antimeningococcus Serum* Intravenously
S② = " " Intraspinously
*Polyvalent
PULSE
SERUM SICKNESS
AUGUST
PULSE
S② 40cc.
SERUM SICKNESS

the feces was negative. A dental consultation revealed three impacted molar teeth, and one tooth showing periapical infection.

Course in Hospital.—During the first three days of his stay in the hospital the patient was afebrile and there were no striking developments. The only complaints were of lassitude and slight headache. On the fourth day the temperature rose to 101° F. and headache and joint pains were complained of. No chills or sweats developed. A laryngologist advised the removal of the tonsils, feeling that, in the light of the negative findings elsewhere, they might explain his condition. On July 13 there appeared on the dorsum of the right hand a small nodule approximately 1 cm. in diameter, elevated and presenting a red center. It was quite tender. A similar nodule appeared simultaneously on the dorsum of the right foot. There were no petechiæ or other skin manifestations.

On July 15 the patient was transferred to the surgical service for tonsillectomy. This was performed under general anæsthesia, and the patient made an uneventful recovery from the operation.

On July 25 the patient was readmitted to the medical service. During his ten-day stay in the surgical ward there had been no improvement. Fever, pains in the joints, malaise and general weakness persisted. On this admission examinations revealed no new development other than a heavy trace of albumin in the urine. An x-ray plate of the paranasal sinuses was reported normal. Blood cultures, made on July 26 and 27, were sterile.

On July 31 lumbar puncture was performed and 10 c. c. of clear limpid fluid, under slightly increased pressure, were removed. The cell count was 30 per c. mm., and there was a positive globulin test. The colloidal gold curve was normal, and the Wassermann test negative. Cultures of this fluid and of the blood, taken on the following day, were negative. The headache was somewhat relieved by the lumbar puncture.

On August 3 a few bright red, maculopapular skin lesions were noted and another blood culture was made (the sixth since his admission to the hospital). This culture was reported, three days later, as showing a meningococcus, "paranormal" type. This report was confirmed by a second positive blood culture taken on August 6. Specific anti-meningococcus treatment (intravenous and intraspinous) was immediately instituted.

August 7.—Forty cubic centimeters of polyvalent antimeningococcus serum were given intravenously and 35 c. c. subthecally. The cerebrospinal fluid, withdrawn before the introduction of the serum into the spinal canal, was clear, contained 48 cells per c. mm., and gave a positive globulin test. The Wassermann test and colloidal gold curve were negative. The fluid was sterile.

August 8.—Forty cubic centimeters of antimeningococcus serum (polyvalent) were injected intraspinously. There was moderate headache and the temperature reached 100° F. (maximum for the day). His general condition seemed better. The cerebro-spinal fluid was sterile.

August 9.—Thirty-five cubic centimeters of antimeningococcus serum were injected intraspinously and 40 c. c. intravenously. The cell count of the fluid removed at the time of the intraspinous treatment was 315 per c. mm. It was negative on culture. There was slight stiffness of the neck, and Kernig's sign was present, but the patient had become afebrile and his general condition was distinctly improved.

August 10.—Forty cubic centimeters of antimeningococcus serum were given intraspinously. The cerebrospinal fluid cell count was 815 per c. mm.

August 11.—He was much better. Ophthalmoscopic examination was negative. On the following day serum sickness developed, associated with the typical urticarial rash, low intermittent fever, and headache. This persisted for five days.

Following the disappearance of the serum disease, the patient made a rapid recovery. The headaches, skin eruption, joint pains and malaise entirely disappeared and he began to gain in weight rapidly. On August 25 he was discharged to complete his convalescence at his home.

Discussion

Saloman, in 1902, reported the first instance of this type of meningococcus septicæmia. The history and course of the disease in his case were typical and will therefore be briefly summarized here:

A woman, 35 years old, suddenly began to have pains in her joints. The next day a chill followed by fever and malaise developed, and a skin eruption appeared. Four days later she entered the hospital complaining of her joints, and of general weakness. The temperature was 36.6° C. and rose to 39.2° C. It persisted and was septic in type. A purpuric eruption was present and the spleen was palpable. There was a trace of albumin in the urine. On the seventh day of her illness herpes labialis was noted, and a new crop of purpuric and petechial spots appeared. The following day *Diplococcus intracellularis* (*Weichselbaum*) was cultivated from her blood. Some of the skin lesions were nodular in type. After a course of almost two months' duration characterized by chills, high but remittent temperature and multiform erythematous skin eruptions, a convulsion occurred with the subsequent development of symptoms and signs of cerebrospinal meningitis. The specific organism was recovered from the cerebrospinal fluid. After a stormy course the temperature finally reached normal on the 120th day of the disease, and the patient made a good recovery.

Cases similar to the above have been reported by Netter (1903); Rist and Paris (1904); Martini and Rhodes (1905); Liebermeister (1908); Bovaird (1909); Lemierre, May and Portret (1912); Sakai; Ottinger, P.-L. Marie and Baron (1913); Bray; Sainton and Maille; Kohlisch (1915); Netter; P.-L.Marie, Zeissler and Reidel (1917); Ribière, Herbert and Bloch (1919); Sergent and Bordet (1920) and others. There is a remarkable similarity in these reports, which has led some French writers on the subject to conclude that the condition can be diagnosed at the bedside, without the help of laboratory findings. The clinical features emphasized will be hastily reviewed at this point.

The history is usually that of a disease of sudden onset, characterized by chills, fever and malaise. Drenching sweats may occur. Headache is common. There may be nausea and vomiting at the onset, though gastro-intestinal symptoms are not prominent. As the condition progresses, weakness, irritability and anorexia ensue, and the patient, if not forced to do so at the beginning, soon takes to his bed. Joint and muscle pains are common, and a multiform erythematous eruption almost invariably appears. In the latter condition the dominant finding is a raised, firm, nodular, red or bluish-red lesion varying in size from 0.25 to 2 cm. in diameter, and usually tender. These lesions appear in crops and gradually disappear, only to recur in the course of a few days.* The temperature curve varies in type though it is characteristically of a rather high but remittent or intermittent variety, with occasional days of entire freedom from fever. Netter particularly

* Renault and Cain have studied similar lesions occurring in nine cases of cerebrospinal meningitis with meningococcus septicæmia. The major changes occurred in the blood vessels and in the neighborhood of the capillaries. The presence of the meningococcus here was proven both microscopically and culturally.

called attention to the remittent type of the febrile reaction, and this has been noted by others, especially Lancelin, Bour, Sevestre and Hutinel. The chart may be very similar to that of a tertian or quartan malarial infection.

On examination, the patient presents the usual picture of a mild type of sepsis, with certain modifications. The skin manifestations, as mentioned above, are prominent, and form the most constant and characteristic clinical feature of the disease. Attention has been called by many observers, more especially by Netter, to the association of erythema nodosum with cerebrospinal meningitis and meningococcus septicæmia.

There is often a slight general glandular enlargement, although this is by no means a constant finding. The occurrence of an iridocyclitis was reported in one case.

A mild bronchitis is frequently present, though its occurrence is not so constant here as in typhoid fever, with which meningococcæmia has been confused. The heart shows nothing. It is interesting that a few cases associated with pulmonary tuberculosis have been reported. (Pissavy, Richet the younger and Pignot; Combe, Léon and Blaye; Bray.) The pulse is usually rapid in proportion to the height of the fever although a relative bradycardia may occur (suggestive of meningeal involvement). A firm spleen is frequently made out early in the course of the disease and the edge is invariably palpable below the costal margin after the affection has persisted for some time. The joints are often tender and may show a considerable limitation of motion. An actual purulent arthritis is very uncommon, although it may occur. Simple arthralgia with slight limitation of motion is the usual finding. Signs of involvement of the central nervous system are entirely absent in the premeningitic stage. As has been pointed out, however, those cases which do not receive specific antimeningococcus therapy are prone to develop a typical cerebrospinal meningitis, with the usual symptoms and signs. Examination of the blood usually reveals a well-marked leukocytosis (15,000 to 25,000), with a relative and actual polymorphonuclear increase. The latter, however, is not constant. A mononucleosis may occur (Ottinger, P.-L. Marie and Baron) and in the absence of an outspoken leukocytosis, this may lead to errors in diagnosis (typhoid fever, tuberculosis). The urine is usually concentrated, and contains a trace of albumin with an occasional hyaline or finely granular cast. Examination of the cerebrospinal fluid may be entirely negative, or may reveal a moderate pleocytosis and a positive globulin test. Cultures of the fluid are, of course, negative in the "premeningitic" stage.* As will be mentioned later, the presence of a positive globulin test and a high lymphocytosis in many of these cases, has led some observers to believe that the focus, from which the organism is disseminated into the general circulation, is situated at least in the proximity of the central nervous system. Cultures of the blood reveal the causative agent of the disease.

The most characteristic single clinical finding in the cases reported has been the painful erythematous nodules, which appear in crops, and which, on disappearing, do not leave the well-marked bluish discoloration of the skin so constant in erythema nodosum. The presence of these lesions, together with arthralgia or myalgia, is highly suggestive, when occurring in a patient running a prolonged septic course, and in whom the commoner causes of long continued, irregular or intermittent fever have been excluded.

However, a definite diagnosis of chronic meningococcus septicæmia can be made only after the presence of the organism in the circulating blood has been demonstrated by culture.†

If, during the course of a prolonged septic fever accompanied by the phenomena mentioned above, evidences of meningitis appear, one may be almost certain that a chronic meningococcæmia has been present, and that "localization" of the organisms in the meninges has occurred.

Treatment

Every effort should be directed toward the immediate sterilization of the blood stream before the infection has had time to become localized in the meninges, and thus give rise to an acute cerebrospinal meningitis.

Once the diagnosis is made, intensive intravenous antimeningococcus serum therapy should be started, due precautions against untoward serum reactions having been observed.

The question of subthecal treatment is an important one, and has been discussed at length by Ribierre, Herbert and Bloch in their article on this subject. According to these authors there are two definite indications for intraspinous serum therapy: (1) Evidence suggestive of meningeal localization of the organism, that is, signs of meningitis (increased irritability, rigidity of the neck, Kernig's sign, increased reflexes, an increased cell count in the cerebrospinal fluid or the recovery of the organism from the fluid). (2) Cases which do not respond promptly to treatment solely by the intravenous route.

Under the first group fall two classes of cases, (a) those with frank signs of an acute meningococcus meningitis, (b) those with only slight or inconspicuous evidence of actual meningeal

* Lumbar puncture in cases of suspected and proven meningococcus septicæmia may not be devoid of danger. It should be practised only in the presence of meningeal symptoms, or as a means of providing an additional route for the administration of serum. Weed, Wegeforth, Ayer and Felton found that release of cerebrospinal fluid during certain *artificial* septicæmias in animal experiments resulted in the meningeal localization of the organism. Wegeforth and Latham report two cases of pneumococcus septicæmia; in one of these lumbar puncture was performed on account of the presence of meningitic symptoms; in the other these symptoms were absent, but the procedure was carried out for the purpose of completing the clinical study of the case. In both instances normal spinal fluid was obtained at the initial puncture. Meningitis subsequently developed in each case.

† The medium employed (agar-agar or bouillon) should be enriched by the addition of hydrocele, ascitic or pleural fluid, approximately 1 c. c. of fluid for each 10 c. c. of medium. 0.5 per cent glucose may be added. The bouillon should be incubated for half an hour before inoculation. Blood should be drawn during the chill, or at the beginning of the daily rise of temperature, and the medium immediately inoculated, preferably at the bedside.

infection. The former should be treated as ordinary cases of cerebrospinal meningitis, the major therapy being directed to the local condition (*i. e.,* the meningitis). Here, as in the epidemic form of the disease, the usual occurrence is for the bacteræmia to disappear with the development of the meningitis. However, should the meningococcæmia persist, both intravenous and subthecal administration of the anti-serum is indicated. In the second type (b) headache, photophobia, slight muscular rigidity, a suggestive Kernig's sign and somewhat heightened reflexes may occur. The cerebrospinal fluid is clear and its pressure not greatly increased. The cell count may be high (30 per c. mm. in our case) but the cells are mainly lymphocytes. A positive globulin test may be present. The fluid, however, is sterile on culture. It is this combination of clinical and laboratory findings that has led to the assumption by some observers that the primary focus of the infection is situated at a point in close proximity to the central nervous system. They do not, however, regard these phenomena as evidence of an actual meningeal infection. Regardless of the cause, the therapeutic indications seem clear in the presence of these findings. Such patients should be treated by subthecal injections of the anti-serum, either as a curative or a prophylactic measure.

A few uncomplicated cases in the literature have responded very slowly to the intravenous treatment alone, and it is urged by members of the French school that they be treated intraspinously as well, even in the absence of any evidence of meningeal irritation. These authors base their reasoning on the belief that the focus from which the organism is disseminated into the blood stream is located near the central nervous system, and that intraspinous therapy together with intravenous therapy offers the logical approach to this focus. Although this question did not arise in the treatment of our two cases (one having developed a frank meningitis, and the other showing evidences of meningeal irritation, before specific serum therapy of any sort was instituted), no hesitancy would have been felt in resorting to the intraspinous route as an aid to the treatment of pure meningococcus septicæmia without central nervous system manifestations.

The treatment of complications of the disease other than the meningitis will not be discussed here. They are dealt with as in epidemic and sporadic cases of cerebrospinal meningitis.

Serum.—Authorities urge the immediate classification of the organism (normal, paranormal or irregular; Type I, II, III or IV of Gordon; Type A, B or C of Nicolle) in order that monovalent serum may be used. When delay occurs in determining the type, temporary treatment with a polyvalent serum may be instituted. In both of the cases here reported (one an infection with a "normal," and the other with a "paranormal" strain) the monovalent serum treatment was not feasible at the time. Satisfactory results were obtained by the use of a polyvalent anti-meningococcus serum furnished by the New York State Board of Health.

Dosage.—Daily intravenous injections of from 20 to 40 c. c. of serum are advocated. In most of the reported cases from three to five treatments sufficed. When intravenous treatment is contemplated, an equal amount may be given by this route.

Ribierre, Herbert and Bloch advocate the following procedure in the treatment of an uncomplicated case of chronic meningococcæmia: Type the causative organism immediately, and use the specific anti-serum as soon as possible. Until this is available a polyvalent serum should be employed. The treatment is begun with the daily injection of 40 c. c. of anti-meningococcus serum on one day subcutaneously and on the next intramuscularly, until five treatments have been given. If no immediate improvement results, the intravenous route should be utilized. If this fails, the serum should be given subthecally.

Vaccine Therapy.—Sergent and Provost report a case of chronic meningococcus septicæmia in which intravenous therapy (in all 100 c. c.) was without curative effect, and in which intraveneous vaccine treatment was finally employed. The patient recovered promptly. Adherents to this form of therapy, and especially Boidin, advocate also the production of an "abscess of fixation" as a definite curative measure, successful in cases in which all other forms of therapy produce no results.

In both of our cases, inasmuch as the patients were promptly cured by intravenous and intraspinous serum treatment, the other procedures recommended were not employed. From a careful review of the literature it seems that a large majority of cases respond in a similarly prompt and satisfactory manner, and that it is only the rare exception which demands other measures.

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PERNICIOUS ANEMIA

A CLINICAL STUDY OF ONE HUNDRED AND FIFTY CONSECUTIVE CASES WITH SPECIAL REFERENCE TO GASTRIC ANACIDITY

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This study was begun in 1915. Our interest was aroused at that time because we observed in all our cases of pernicious anemia an absence of free hydrocholoric acid in the gastric contents. For a long time it has been known that the absence of free hydrochloric acid is a frequent finding in this disease, although the actual incidence in the reports of various authors has differed considerably. In isolated cases studied over long periods of time, the achylia gastrica antedated the development of the anemia by several years. Thus K. Faber[1] noted achylia gastrica in three cases, eight, four and ten years before death from pernicious anemia. In each instance when the achylia was first observed the hemoglobin was over 90 per cent, and in each case the patient developed the picture typical of pernicious anemia long after the finding of anacidity. Very recently Friedenwald and Morrison[2] have given a good review of the conflicting evidence concerning the character of gastric secretion in pernicious anemia; in 74 per cent of their own 57 cases achylia was noted. The problem to be solved was whether, in arriving at the diagnosis, the presence or absence of free hydrochloric acid was possibly of as much value as some of the other acknowledged factors, such as the high color index of the blood or the presence of macrocytes in the smear.

We decided, therefore, upon a routine gastric analysis in all cases of possible pernicious anemia, and further, upon the repetition of the analysis during the progress of the disease, especially during remissions.

To obtain these data the Rehfuss tube was introduced. The fasting contents were taken; the tube was retained, an Ewald test meal given and samples taken at forty-minute intervals over a varying period of time. In a few cases, a test was made for the presence of pepsin.

We have observed in the clinic of this hospital 150 consecutive cases of pernicious anemia. We are including in this paper a brief discussion of several other points which we believe to be of sufficient interest to warrant this inclusion, namely, a discussion of seven special cases, the family history, racial discrepancies, syphilis and pernicious anemia, the occurrence of eosinophilia, the typical blood picture, unnecessary abdominal operations, the occurrence of gray hair and age and sex considerations.

The Gastric Secretion in Pernicious Anemia

As may be seen from Table 1, of the 143 cases of pernicious anemia with proved diagnosis, in 107 a gastric analysis was made one or more times. In a few of the cases the analyses cover a period of years. In one instance they were made on samples taken every 15 minutes for 2 hours and 30 minutes. In all but three cases there was no free hydrochloric acid. Hence it may be assumed that, as a rule, anacidity is constant and persistent throughout the disease. In the cases of five patients pepsin was absent in the seven analyses made. Since this series was completed pepsin has been found absent in most of the cases examined. Further data are being obtained on this point.

In the three cases in which some free hydrochloric acid was demonstrated, the following points are worth noting:

1. In case 58, there was a perfectly normal acidity but the post-mortem examination corroborated the diagnosis of pernicious anemia.

2. In case 117 the picture presented was typical of pernicious anemia. The patient had undergone three operations for a persistent bloody diarrhœa at one of which, nine years before admission, eleven inches of intestine had been removed. There was much difference of opinion as to the correct diagnosis. Finally "atypical anemia, primary anemia (?)" was agreed upon.

3. In case 124 the symptoms pointed to the presence of a tumor of the spinal cord. A subsequent operation disproved this assumption. Death followed one month later. In this instance, the first gastric analysis showed complete anacidity in all samples but the fourth, which gave a free HCl of 6.

TABLE 1

Serial number	Hospital number	Clinical diagnosis	Age	Sex	Wassermann	Birthplace	Eosinophilia, per cent	Gastric analysis *	Date of gastric analysis	Hemoglobin, per cent †	Family history	Tongue ‡	Hair §	Date of admission	Blood smear ‖	Follow-up notes
1	1731	Posterior-lateral sclerosis. Pernicious anemia.	46	M.	—	U. S.	1.5	? M 0 ? M 12	10–14–14	84	Neg.	T.	?	10–6–14	S.	No note.
2	2133	Pernicious anemia........	68	F.	—	Canada.	1	?	....	?	Tbc.	N.	?	1–5–15	T.	No note.
3	1548	Pernicious anemia........	41	F.	—	U. S.	18	?	....	?	Neg.	T.	?	8–18–14	T.	Splenectomy. Died, 3–20–16.
4	2282	Pernicious anemia........	63	M.	—	U. S.	9	0 M 0 1 M 4	2–6–15	29	Neg.	T.	G.	2–5–15	T.	
	4067	Arteriosclerosis........	......	......	......	....	10	0 M 0–0–0 0 M 4–14–12	1–30–16	30	....	......	......	1–9–16		Died, 6–15–17.
5	2049	Secondary anemia.......	57	M.	—	U. S.	5.5	? M 0 M 1	12–27–14	40	Neg.	?	?	12–16–14	S.	
	2437	Pernicious anemia. Arteriosclerosis.	......	......	......	....	......	? M 0 M 5	3–8–15	32	....	......	......	3–5–15	T.	Died, October, 1915.
6	2808	? Pernicious anemia. Chronic arthritis of spine.	42	M.	—	U. S.	3	0 M 0	5–25–15	80	Neg.	?	?	5–18–15	S.	Died, January, 1917.
7	2848	Pernicious anemia. Chronic nephritis.	56	M.	—	U. S.	4	?	?	?	Neg.	?	W.	5–25–15	T.	January, 1917, working.
8	4181	Pernicious anemia........	51	M.	—	Russia.	0	0 M ? 10 M	2–24–16	33	Neg.	T.	G.	2–17–16	T.	
								0 M 0–0–0 12 M 12 15–18 No pepsin.	5–7–16	82						
							5	0 M 0–0 5 M 4–4 No pepsin.	10–2–17	55	....	......	......	....	......	Died, 8–13–18.
9	4316	Pernicious anemia........	48	F.	—	Canada.	3	0 M 0–0–0 8 M 8–14–10 No pepsin.	3–16–16	52	Neg.	T.	?	3–13–16	T.	Died, June, 1916.
								0 M 0–0–0 13 M 9–11–13 No pepsin.	5–21–16	67						
10	4404	Pernicious anemia........	60	F.	—	U. S.	4	0 M 0–0–0 12 M 20–15–34 No pepsin.	3–29–16	30	Cancer. Tbc.	T.	W.	3–27–16	T.	Died, 6–28–16. P. M., Pernicious anemia.
11	4565	Pernicious anemia........	38	F.	—	Canada.	8	0 M 0 8 M 5	8–9–16	88	Neg.	T.	?	4–26–16	T.	October, 1920, working; doing very well.
12	4719	Pernicious anemia........	73	F.	—	U. S.	0	?	....	?	Neg.	T.	G.	5–24–16	T.	Died, 6–10–16. Pernicious anemia.
13	4609	Pernicious anemia........	73	F.	—	U. S.	0	0 M 0–0 15 M 9 12	5–5–16	29	Cancer.	S.	W.	5–4–16	T.	
								0 M 0	7–3–16	37	....	......	......	....	......	Died, November, 1916.
14	4748	Pernicious anemia........	42	M.	—	U. S.	3	?	....	?	Tbc.	T.	G.	5–29–16	T.	Died, 7–27–16. P. M., Pernicious anemia.
15	4649	Pernicious anemia........	63	M.	—	U. S.	4	? M 0 0–0 M 4–8–15	5–22–16	35	Neg.	T.	G.	5–18–16	T.	
								0 M 0 8 M 5	9–1–16	104	....	......	......	....	......	Died, 12 26–16.
16	42	Pernicious anemia........	44	F.	?	England.	0	?	....	?	Neg.	?	?	4–25–13	T.	No note.
17	1169	Pernicious anemia........	35	F.	—	U. S.	0	?	....	?	Neg.	S.	N.	5–7–14	T.	Doubtful diagnosis, Pernicious anemia.
		Purpura	......	......	......	....	......	....	....	.....	....	......	......	....	......	January, 1920, came in for bleeding from varicose vein.
18	1132	Pernicious anemia. Cancer of stomach.	44	M.	—	Sweden.	4	? M 0 M 24	5–7–14	32	Cancer. Tbc.	?	?	4–29–14	T.	X-ray showed definite cancer of stomach. Two diagnoses here. Died; date unknown.
19	1324	Pernicious anemia. Acute pericarditis.	51	F.	—	U. S.	5	0 M 0 0 M 13	9–22–16	111	Neg.	T.	W.	6–9–14	T.	
	10380	....	......	......	......	....	......	0 M 0–0–0 0 M 8–10–8	1–25–19	39	....	......	......	1–24–19	......	Died, April, 1919.
20	1327	Pernicious anemia? Cancer of stomach?	54	F.	—	U. S.	0	? M 0 M 1	7–5–14	78	Tbc.	?	W.	8–18–14	At.	May, 1915, hemoglobin, 95%.
21	1544	Pernicious anemia........	44	M.	—	U. S.	6	?	?	?	Neg.	T.	W.	8–17–14	T.	Died, August, 1916.
22	2789	Pernicious anemia ?......	32	F.	—	Canada.	0	?	....	?	Neg.	?	?	5–14–15	T.	9–29–16, hemoglobin, 121%; smear normal. October 10, 1920, doing fairly well.
23	2788	Pernicious anemia........	49	F.	—	Canada.	2	? M 0 M 4	5–14–15	35	Neg.	T.	?	5–13–15	T.	Splenectomy. Died, March 28, 1918.
24	3045	Pernicious anemia; syphilis. Mitral stenosis. Acute endocarditis.	31	M.	+ –	U. S.	7	?	....	?	Neg.	T.	?	7–5–15	T.	Died, 5–7–17. P. M., Pernicious anemia, mitral stenosis, acute pericarditis.
	6395	....	......	......	......	....	0	0 M 0–0–0 4 M 12–8–4	4–8–17	34	....	......	......	4–5–17	......	Acute peritonitis.
25	3095	Pernicious anemia. Asthma. Chronic nephritis?	49	M.	—	U. S.	17	?	....	?	Tbc.	T.	G.	7–17–15	T.	Died, 12–7–15.
26	2984	Pernicious anemia........	47	F.	—	U. S.	24	0 M 0 M 10	6–23–15	23	Neg.	T.	G.	6–22–15	T.	Died, 12–13–17.
27	3096	Pernicious anemia........	50	F.	—	U. S.	17	0 M 0	8–19–15	40	Cancer.	T.	G.	7–17–15	T.	
							12	0 M 0 6 M 11	10–10–16	70						
	5913	....	......	......	......	....	0	0 M 0–0–0 0 M 6–6–4	1–11–17	20	....	......	......	1–8–17	....	January, 1921. Doctor replies that patient was doing well when last seen, but does not say when.
28	3139	Pernicious anemia........	59	F.	—	England.	16	0 M 0 23 M 18	8–30–15	35	Tbc. Cancer.	T.	W.	7–28–15	T.	10–28–15. Splenectomy.
	4217	....	......	......	......	....	10	0 M 0–0–0 8 M 10–8–8 No pepsin.	2–24–16	36	....	......	......	2–23–16	......	Died, 7–20–16.
29	4935	Pernicious anemia........	55	M.	—	England.	5	0 M 0–0–0 14 M 12–10–8	7–2–16	70	Neg.	T.	W.	7–2–16	T.	Died, 12–26–17.

* Upper figure is the free HCl, lower is the total acid, expressed in c. c. of N/10 HCl per 100 c. c. stomach contents. M indicates test meal.
† Hemoglobin determinations made the same day as gastric analysis or within a few days. ‡ T = typical. S = suggestive. N = normal.
§ G = gray. W = white. N = normal. ‖ T = typical. S = suggestive. At = atypical.

TABLE 1—CONTINUED

Serial number	Hospital number	Clinical diagnosis	Age	Sex	Wassermann	Birthplace	Eosinophilia, per cent	Gastric analysis*	Date of gastric analysis	Hemoglobin, per cent†	Family history	Tongue‡	Hair§	Date of admission	Blood smear‖	Follow-up notes
30	4478	Pernicious anemia........	38	F.	—	Ireland.	5	0 M 0-0-0 8 M 18-20-20	4-12-16	28	Neg.	T.	G.	4- 8-16	T.	
								0 M 0 6 M 20	4-21-17	85						
								0 M 0-0 0 4 M 8 6 6	10- 7-20	70	....	......	......	....	......	October 7, 1920. Tongue smooth. Working.
31	5070	Pernicious anemia........	47	M.	—	U. S.	7	0 M 0-0-0 24 M 20-22-18	8-10-16	67	Neg.	T.	G.	8- 7-16	T.	October 11, 1920. In fair health; has neuritis.
32	589	Chronic nephritis. Hypertension. Arteriosclerosis.	......	......	......	....	......	....	....	......	....	......	......	12- 2-13		
	5169	Pernicious anemia; chronic nephritis; chronic myocarditis. ? Lead poisoning.	56	M.	—	Ireland.	3	0 M 0 8 M 10	9-27-16	67	Tbc.	T.	?	8-21-16	T.	Died, May 8, 1917.
33	5174	Pernicious anemia. Chronic nephritis. Chronic uremia. Insolation.	49	F.	—	England.	0	?	....	?	Neg.	S.	G.	8-22-16	T.	Died, 8-25-16. Terminal temp. 107°. ? Sunstroke. Very hot day. No P. M.
34	4949	Pernicious anemia........	47	M.	—	U. S.	8	0 M 0-0-0 14 M 12-24-14	7-10-16	45	Tbc.	T.	G.	7- 8-16	T.	No note.
35	3435	Pernicious anemia........	48	F.	—	U. S.	7	? M 0-0-0 M 3-15-6	10-12-15	29	Cancer.	T.	N.	10- 8-15	T.	
								0 M 0 5 M 10	9-19-16	61	....	......	......	....	......	Died between May, 1917, and December, 1918.
36	3491	Pernicious anemia........	47	M.	—	U. S.	4	0 M 0 4 M 8	10-24-15	40	Neg.	T.	N.	10-20-15	T.	Sept., 1917. Hemoglobin 112%. Doing heavy work.
								0 M 0-0-0 4 M 10-8	10-30-19	80	....	......	......	....	......	9-6-20. Feels well; treated by a quack.
37	3538	Perniciaus anemia........	54	M.	—	Sweden.	3	M 0 M 8	10-28-15	37	P. A.	S.	G.	10-28-15	S.	
							6	0 M 0 M 14	9-18-16	124	....	......	......	....	......	Died, January 26, 1920.
38	3636	? Pernicious anemia. ? Cancer of stomach.	66	M.	—	Ireland.	9	0 M 0 M 1	11-18-15	50	Cancer.	S.	G.	11-17-15	S.	Atypical case.
								0 M 0 0-0-0 M 3 6 8 10	11-28-15	60	....	......	......	....	......	August, 1917. Hemoglobin, 80%. Cramps in legs. Is working. This discredits diagnosis of cancer.
								0 M 0-0 6 M 5 1	2-11-16	78						
								0 M 0 ? M 9	9-18-16	91	....	......	......	....	......	Died, August 23, 1919.
39	1784	Pernicious anemia........	42	F.	—	Ireland.	6	....	....	?	Neg.	T.	G.	10-19-14	T.	Died, 2-25-16.
40	2834	Pernicious anemia........	31	M.	—	Canada.	1	Free HCl in vomitus, 0	....	......	Neg.	T.	N.	5-22-15	T.	Died, 6-23-15.
41	3768	Pernicious anemia. Hemorrhagic stomatitis.	30	F.	—	U. S.	2	?	....	?	Neg.	N.	N.	12-11-15	S.	Died, ?-15-16.
42	106	Pernicious anemia. Chronic nephritis. ?	59	F.	—	U. S.	6	?	....	?	Cancer.	T.	?	5-23-13	T.	Died, 11-6-15.
43	135	Pernicious anemia........	61	F.	—	U. S.	7	?	....	?	Neg.	?	?	6-11-13	T.	Splenectomy. Died during 1915.
44	780	Pernicious anemia........	44	F.	—	England.	7	?	....	?	Neg.	T.	?	1- 3-14	T.	Died, 2-20-15.
45	881	Pernicious anemia........	61	F.	—	Canada.	6	?	....	?	Tbc.	?	?	2-26-14	T.	Died, 12-6-15.
46	1130	Pernicious anemia........	51	M.	?	U. S.	1	?	....	?	Neg.	?	?	4-29-14	T.	Died, 5-1-14. P. M., aplastic bone marrow.
47	1413	Pernicious anemia........	38	F.	—	U. S.	2	?	....	?	Neg.	?	?	7-10-14	T.	Died, 1-5-15. P. M., pernicious anemia.
48	1410	Pernicious anemia........	72	M.	—	U. S.	1	?	....	?	Neg.	?	?	7-10-14	T.	Died, August 31, 1916 (or 1915).
49	1280	Pernicious anemia. Hemophilia.	26	M.	—	U. S.	4	?	....	?	Neg.	?	?	6- 3-14	T.	Died, 9-2-14.
50	2680	Pernicious anemia. Eosinophilia, cause unknown.	64	F.	—	U. S.	28	0 M 0 M 14	5- 8-15	25	Neg.	S.	?	4-23-15	T.	No note.
51	3063	Pernicious anemia. Exhaustive psychosis.	51	F.	—	Canada.	3	Vomitus No. M 0 Free HCl M 6	7-11-15	36	Cancer.	?	G.	7- 0-15	T.	Died, 6-22-15.
52	3718	Pernicious anemia........	36	F.	—	Canada.	6	0 M 0-0-0-0 M 4 3-5-4	12-11-15	35	P. A.	T.	N.	12- 2-15	T.	
								0 M 0 6 M 9	9-19-16	87	....	......	......	....	......	Died, 1917.
53	3690	Pernicious anemia. Syphilis.	68	M.	++	U. S.	9	0 M 0 0 0 M 22-10-4	11-29-15	21	Neg.	T.	W.	11-27-15	T.	
								0 M 0-0-0 M 2-2-3	1-19-16	93	....	......	......	....	......	Died, Nov. 27, 1916. Received vigorous anti-syphilitic treatment.
								0 M 0 0-0 No pepsin.	3-20-16	72						
54	3863	Pernicious anemia. Syphilis.	43	M.	++	Canada.	8	0 M 0 0-0 8 M 11-11-18	1-13-16	28	Cancer. Anemia.	T.	G.	12-29-15	T.	Received anti-luetic treatment. Died, April, 1917.
								0 M 0 0 M 10	3-21-17	88						
55	4063	Pernicious anemia. Syphilis.	63	F.	++	U. S.	7	0 M 0-0-0 0 M 4 3	1-30-16	48	Anemia.	T.	G.	1-29-16	T.	September 23, 1920. Been very well since leaving hospital.
								0 M 0-0-0 10 M 27-10-10	3- 3-16	90						
56	4430	Pernicious anemia. Chronic Nephritis. Hypertension. Chronic Myocarditis. ? Angina Pectoris.	60	F.	—	U. S.	0	0 M 0 0 0 15 M 20 18 18	4- 1-16	78	Tbc.	S.	G.	3-31-16	S.	Died, 9-6-17. P. M., pernicious anemia, chronic nephritis and postero-lateral sclerosis.
57	4292	Pernicious anemia........	28	F.	—	U. S.	1	0 M 0-0-0 10 M 10-20-15	3-10-16	22	Neg.	T.	G.	3- 8-16	T.	
	5211	....	......	......	......	....	......	0 M 0-0-0 3 M 2 4 4	10-18-16	85	....	......	......	8-28-16	......	Died, May 5, 1917.

* Upper figure is the free HCl, lower is the total acid, expressed in c. c. of N-10 HCl per 100 c. c. stomach contents. M indicates test meal.
† Hemoglobin determinations made the same day as gastric analysis or within a few days. ‡ T = typical. S = suggestive. N = normal.
§ G = gray. W = white. N = normal. ‖ T = typical. S = suggestive. At = atypical.

TABLE 1—Continued

Serial number	Hospital number	Clinical diagnosis	Age	Sex	Wassermann	Birthplace	Eosinophilia, per cent	Gastric analysis *	Date of gastric analysis	Hemoglobin, per cent †	Family history	Tongue ‡	Hair §	Date of admission	Blood smear ‖	Follow-up notes
58	5330	Pernicious anemia. B. pyocyaneus, generalized infection.	58	F.	—	U. S.	0	0 M 22 36-50 22 M 54-60 62	9–22–16	63	Neg.	T.	G.	9–20–16	T.	Died, 10–19–16. P. M., pernicious anemia, fibrous pericarditis and pleuritis. Autopsy stomach contents. No free HCl.
59	5409	Pernicious anemia. Chronic cystitis. Psychosis, symptomatic.	45	F.	—	Ireland.	1	0 M 0 0-0 M 3	10–16–16	20	Neg.	T.	G.	10–7–16	T.	No note.
60	5485	Pernicious anemia........	21	F.	—	U. S.	3	0 M 0-0 0 2 M 6 4 20	11-24-16	56	P. A.	T.	N.	10–23–16	T.	Considered Tbc. at Sharon.
								0 M 0 0 0 M 3 6 13	12–18–16	90	......	......	......	....	......	Died, December 30, 1917.
61	5585	Pernicious anemia........	54	M.	—	U. S.	2	0 M 0 0–0 8 M 14–10 10	11–14 16	80	Tbc.	T.	G.	11–12–16	At.	June, 1918, about the same.
62	5610	Pernicious anemia. Chronic nephritis.	65	F.	—	U. S.	1	0 M 0–0–0 M 14	11–17–16	25	Neg.	T.	G.	11–16–16	T.	Colored. Died during 1917.
63	5657	Pernicious anemia. Chronic nephritis.	62	F.	—	U. S.	0	0 M 0–0–0 6 M 10	11–25 16	30	Neg.	T.	W.	11–14–16	T.	Died, February 26, 1918.
64	5590	Pernicious anemia........	43	F.	—	Canada.	1	0 M 0 0 0 4 M 2 4	11–15–16	73	Neg.	N.	?	11–13–16	at.	Died, April, 1917.
65	5377	Pernicious anemia........	29	M.	—	U. S.	4	0 M 0 0 0 14 M 24 24-12	10– 5–16	20	Neg.	T.	G.	9–30–16	T.	Died, 3–21–17. P. M., Pernicious anemia; acute peritonitis; acute nephritis.
66	5548	Pernicious anemia........	48	M.	—	U. S.	5	0 M 0 0 M 5 No pepsin.	1910	90	P. A.	T.	G.	11– 3–16	T.	Diagnosis: Gastric neurosis, due to overwork and worry, 1910.
								0 M 0 0 0 40 M 70 22-26	11- 5–16	42	......	......	......	....	......	Died, October, 1918.
67	6207	Pernicious anemia. Pulmonary emphysema.	66	F.	—	Ireland.	1	? M 0-0 0 M 6 14	2 27-17	25	Neg.	T.	G.	2 25–17	T.	Died, 3 5–17. No P. M.
68	6142	Pernicious anemia........	38	F.	—	U. S.	8	0 M 0–0–0 10 M 4 4	2–16–17	50	Cancer.	T.	G.	2–14–17	T.	Died September 13, 1918.
69	5811	Pernicious anemia? Addison's Disease? Tbc. Retroperitoneal nodes.	42	M.	—	Sweden.	0	0 M 0–0-0 12 M 14–8-10	12–22–16	70	Tbc.	S.	N.	12–20–16	T.	Atypical case. Died, 4–19–17. P. M., nothing except hyperplasia of mesenteric glands and lymphatics of small intestine.
70	6017	Pernicious anemia........	53	F.	—	Russia.	4	? M 0 M 8	1–25–17	40	?	T.	G.	1–22–17	T.	Had psychosis. No note.
71	6394	Pernicious anemia........	57	M.	—	U. S.	0	0 M 0 0-0 10 M 12–8–8	4– 7–17	28	Neg.	T.	G.	4– 5–17	T.	Died, 4–16–17.
72	6364	Pernicious anemia. ? Combined degeneration of spinal cord.	53	M.	—	Sweden.	1	0 M 0–0–0 10 M 10–25–16	4– 2–17	60	Neg.	T.	G.	3-31–17	T.	Died, May, 1917.
73	1729	Pernicious anemia........	53	F.	—	U. S.	3	M 0	10–11–14	94	Neg.	T.	W.	10– 6–14	N.	Not diagnosed pernicious anemia at this time.
								M 13						3– 1–17	T.	Anacidity found with normal blood; twenty months later had typical pernicious anemia. Died, August, 1919.
74	6679	Pernicious anemia. ? Mitral stenosis and mitral insufficiency.	48	F.	-	U. S.	0	?	....	?	Cancer.	T.	W.	5–25–17	T.	Died before August, 1918.
75	6632	Pernicious anemia. Furunculosis.	74	M.	—	Germany.	3	0 M 0-0 0 16 M 12 12-16	5–26–17	40	Neg.	T.	G.	5–18–17	T.	Atypical case, white count of 14,000. X-ray showed filling defect of lesser curvature. Died, November 14, 1917.
	6951	....	......	......	......	....	......	0 M 0-0 5 M 6-0	7–30–17	30						
76	6600	Pernicious anemia. Fractured rib.	46	F.	+ -	Ireland.	4	0 M 0–0–0 10 M 10	5–14–17	40	Neg.	S.	G.	5–12–17	T.	Treatment: diarsenol. June 19, 1920, fairly well.
77	5990	Pernicious anemia. Chronic nephritis.	53	M.	—	U. S.	4	0 M 0-0 0 6 M 4-6	1–20–17	28	Tbc.	T.	G.	1–19–17	T.	Died, 8–12-17.
78	7005	Pernicious anemia. Combined degeneration of spinal cord.	57	M.	—	U. S.	8	0 M 0 0-0 0 0 M 10–6–6	7–31–17	60	Tbc.	T.	G.	7–30–17	T.	Died, May 29, 1920.
79	7072	Pernicious anemia. Mitral stenosis; mitral insufficiency.	42	M.	—	Ireland.	1	0 M 0-0 25 M 17-22	8–16-17	31	Neg.	T.	G.	8–14–17	T.	Died, about June, 1918.
80	7202	Pernicious anemia........	65	M.	—	England.	5	0 M 0 0 0 9 M 12-13-13	9–10–17	46	Neg.	T.	W.	9– 8-17	T.	Died suddenly, 10–2–17. Delirious. P. M., pernicious anemia and pachymeningitis.
81	7256	Pernicious anemia. Mitral stenosis; mitral insufficiency. Paranoid psychosis.	40	F.	—	Sweden.	3	?	....	?	Cancer.	?	N.	9–20–17	T.	9–21–20, no note.
82	7160	? Pernicious anemia. Combined degeneration of spinal cord.	44	M.	—	Canada.	3	.	....	?	Neg.	T.	N.	8–30–17	S.	Died, October, 1917.
83	6974	Pernicious anemia. Arteriosclerosis.	59	F.	—	U. S.	6	0 M 0–0–0 10 M 16–12-14	7–25–17	45	Neg.	S.	G.	7–23–17	T.	Died, 4–26–18.
84	7110	Pernicious anemia........	46	M.	—	U. S.	2	0 M 0-0-0 13 M 14–13-24	8–21–17	29	Tbc.	N.	G.	8–20–17	T.	Died, about September, 1918.
85	6882	Pernicious anemia........	51	M.	—	U. S.	9	0 M 0–0 0 M 9–16–13	7– 7–17	30	Neg.	N.	G.	7– 5–17	T.	Died, December 30, 1918.
86	7207	Pernicious anemia........	58	F.	—	U. S.	3	0 M 0–0 3 M 12 12	9–10–17	49	Neg.	T.	G.	9– 8–17	T.	Died, latter part of 1919.
87	7434	Pernicious anemia. Lateral sclerosis.	40	F.	+	U. S.	4	0 M 0 0 22 M 20 20	10–22–17	68	P. A.	N.	N.	10 20–17	T.	Died, June 3, 1918.
88	7026	? Pernicious anemia......	45	F.	—	Ireland.	4	0 M 0–0–0 0 M 10–0	8– 8–17	45	Neg.	T.	G.	8– 6–17	S.	9–21–20. Was in fair health in spring of 1920.
89	7493	Pernicious anemia. Internal hemorrhoids.	58	M.	—	U. S.	2	0 M 0–0–0 4 M 5–6–6	11 3-17	50	Neg.	N.	G.	11– 1–17	T.	Died, October 14, 1920.
90	7745	Pernicious anemia ?......	65	F.	—	U. S.	0	0 M 0–0–0 5 M 4–4 5	12–27–17	65	Tbc.	T.	G.	12–22–17	S.	Sept., 1920. Living, but in poor health. Unable to use legs much.
91	7719	Pernicious anemia? Lateral sclerosis?	35	F.	—	U. S.	4	?	....	?	Neg.	S.	G.	10–17–17	S.	March 20, 1919. Condition about the same.
92	7736	Pernicious anemia........	29	F.	—	Canada.	8	?	....	?	Neg.	N.	N.	12–20–17	T.	Died, May 30, 1918. Mentally confused, with ataxic paraplegia.
93	8220	Pernicious anemia........	58	M.	—	U. S.	0	0 M 0 0-0 15 M 17 18 20	3 5-18	48	Tbc.	T.	W.	3– 4–18	..	Died, end of March, 1918.

* Upper figure is the free HCl, lower is the total acid, expressed in c. c. of N/10 HCl per 100 c. c. stomach contents. M indicates test meal.
† Hemoglobin determinations made the same day as gastric analysis or within a few days. ‡ T = typical. S = suggestive. N = normal.
§ G = gray. W = white. N = normal. ‖ T = typical. S = suggestive. At = atypical.

TABLE 1—Continued

Serial number	Hospital number	Clinical diagnosis	Age	Sex	Wassermann	Birthplace	Eosinophilia, per cent	Gastric analysis*	Date of gastric analysis	Hemoglobin, per cent †	Family history	Tongue ‡	Hair §	Date of admission	Blood smear ‖	Follow-up notes
94	8217	Pernicious anemia........	56	F.	—	U. S.	5	0 M 0–0–0 M 17–23–13	3– 5–18	35	Neg.	S.	G.	3– 4–18	T.	Died, May 28, 1918.
95	8192	Pernicious anemia........	53	M.	—	U. S.	0	0 M 0–0–0 17 M 9–6–6	3– 6–18	40	Neg.	S.	N.	2–28–18	T.	Died, about May, 1918.
96	8540	Pernicious anemia........	61	M.	—	U. S.	3	? M 0–0–0 M 9–7–12	4–17–18	50	P. A.	T.	G.	4–20–18	T.	
								0 M 0–0–0–0–0 –0–0–0–0–0 M 6–6–5–6–5 –5–12–12 –12–8 Every fifteen minutes.	3–24–19	29	....	......	......	3– 5–19	..	Died, October 28, 1919.
97	8448	Pernicious anemia........	62	F	—	U. S.	4	0 M 0–0 8 M 6	4–11–18	30	Cancer.	N.	W.	4– 9–18	T.	8–30–20. Fine; smear normal.
98	8865	Pernicious anemia........	59	M.	—	U. S.	2	?	....	?	Neg.	?	W.	6–23–18	S.	Died, 6–27–18. P. M., Pernicious anemia and healed Tbc.
99	8817	Pernicious anemia........	62	M.	—	U. S.	4	?	....	?	Tbc.	T.	G.	6–13–18	T.	Died, October 9, 1918.
100	8913	Pernicious anemia........	48	F.	—	England.	7	0 M 0–0–0 8 M 10–11–14	7–10–18	85	Neg.	T.	G.	7– 3–18	S.	Died, May, 1919.
101	9017	Pernicious anemia. Chronic myocarditis. Hypertension.	71	F.	—	U. S.	3	? M 0 0–0	8–22–18	60	Neg.	T.	W.	7–22–18	T.	Died, spring of 1919.
102	9102	Pernicious anemia........	54	F.	—	England.	5	0 M 0–0 0 M 2–2–5	8– 8–18	23	Neg.	N.	W.	8– 9–18	T.	Died, 2–21–19. P. M., Pernicious anemia and chronic nephritis.
103	9291	Pernicious anemia........	63	F.	—	U. S.	1	0 M 0	9–17–18	20	Cancer.	N.	G.	9–16–18	T.	Died, February 21, 1919.
104	9774	Pernicious anemia. Gas bacillus infection; terminal.	41	M.	—	U. S.	1	?	....	?	Neg.	N.	N.	10–30–18	At.	Died, 11–5–18. P. M., Pernicious anemia and gas bacillus infection.
105	9792	Pernicious anemia........	52	M.	—	Ireland.	6	0 M ? 0–0 20 M 16–20	11– 9–18	40	Neg.	S.	G.	11– 5–18	T.	Is a rubber worker and exposed to chemical fumes. April 17, 1920, feels fair. Hemoglobin 60%. ? Anemia, due to chemicals.
106	9900	Pernicious anemia........	33	F.	—	U. S.	0	0 M 0–0–0 8 M 2 10 3	11–23–18	55	P. A.	T.	N.	11–21–18	T.	September, 1920. Quite well. Working.
107	10295	Pernicious anemia........	54	M.	—	Canada.	1	0 M 0 ? 0 5 M 0 12	1–16–19	18	Neg.	T.	G.	1–14–19	T.	Died some time before June, 1920.
108	10324	Pernicious anemia. ? Tuberculous pleurisy.	49	M.	—	U. S.	3	?	....	?	Neg.	T.	G.	1–18–19	T.	Died, 2–14–20.
109	8345	Pernicious anemia........	39	F.	—	Denmark.	4	0 M 0–0–0 2 M 5–6–18	4–27–18	75	Cancer.	S.	G.	3–26–18	T.	
								0 M 0–0–0 10 M 40–0–10	1–25–19	..	....	..	..	1–18–19	..	Died, January, 1920.
110	10748	Pernicious anemia........	47	F.	—	U. S.	1	0 M 0–0–0 20 M 16–0–10	3–28–19	29	Neg.	T.	W.	3–26–19	T.	September, 1918. Dr. F. T. Lord found blood normal. Died Oct. 23, 1919.
111	10630	Pernicious anemia. ? Cholelithiasis.	71	M.	—	U. S.	5	0 M 0–0–0 5 M 50–60	3– 8–19	65	Neg.	S.	W.	3– 6–19	S.	1–7–20. Hemoglobin, 90%. Good condition. October, 1920, in fair health.
112	10691	Pernicious anemia........	36	F.	—	Sweden.	3	0 M 0–0–0 22 M 8–6–6	3–18–19	31	Neg.	T.	N.	3–17–19	T.	No note.
113	10705	Pernicious anemia........	61	F.	—	Alsace Lorraine.	5	0 M 0 M 4	3–20–19	54	Neg.	N.	G.	3–19–19	T.	Died, June 23, 1919.
114	10635	Pernicious anemia........	59	F.	—	U. S.	8	0 M 0 4 M	3–10–19	24	Neg.	S.	G.	3– 6–19	T.	Died, 4–10–20. P. M., Pernicious anemia.
115	11005	Pernicious anemia........	71	F.	—	Canada.	0	0 M 0–0	5– 5–19	17	Tbc.	T.	W.	5– 3–19	T.	Died, May 24, 1919, of pernicious anemia.
116	10939	Pernicious anemia. Combined degeneration of spinal cord.	36	F.	—	U. S.	0	0 M 0–0–0 6 M 4 4 4	4–26–19	51	Neg.	N.	N.	4–23–19	S.	Died, 10–30–19.
117	10872	Anemia (severe, atypical, ? pernicious); chronic diarrhea.	38	F.	—	Canada.	1	4 M 28 30–14 28 M 56–60–34	4–13–19	63	Neg.	T.	N.	4–12–19	T.	Atypical case. Nine years ago 11 inches of gut removed; two other operations for bloody diarrhea.
								0 M 24 0 0 4 M 58–20–8	4 23 19	70						
118	10811	Pernicious anemia. Paralysis of facial nerve.	52	M.	—	Canada.	10	0 M 0–0–0 10 M 8–16–10	4 8–19	22	Tbc.	S.	G.	4– 4–19	T.	July 9, 1919. Hemoglobin 80%. Well. Died, 1–26–20.
119	11201	Pernicious anemia........	43	M.	—	U. S.	3	0 M 0–0–0 6 M 13 10–10	3 12–17	93	Neg.	N.	G.	6– 4–19	T.	Died, 6–5–19. P. M., Pernicious anemia, postero-lateral sclerosis and healed pulmonary tuberculosis.
120	11408	Pernicious anemia........	48	F.	—	U. S.	0	0 M 0–0–0 25 M –10–25	7– 9–19	55	Neg.	N.	G.	7– 8–19	T.	Died, July 23, 1920.
121	11503	Pernicious anemia. ? Chronic myocarditis.	54	M.	—	U. S.	1	0 M 0–0–0 7–12–4–3	7–26–19	35	P. A.	T.	G.	7–24–19	T.	Died, June, 1920.
122	11822	Pernicious anemia........	58	F.	—	Italy	3	0 M 0–0–0 14 M 22–8–2	9–12–19	45	Cancer.	T.	N.	9–11–19	T.	September, 1920. Condition the same.
123	11527	Pernicious anemia. Subacute infectious arthritis.	45	F.	—	Russia.	9	0 M 0–0–0	8– 1–19	85	Neg.	N.	G.	7–29–19	T.	
	13771	....	......	......	......	....	......	0 M 0–0–0 10 M 10–5	6–16–20	65	....	......	......	6–14–20	......	No note.
124	11502	? Tumor of spinal cord. ? Pernicious anemia.	63	M.	—	Canada.	0	0 M 0–0–0 10 M 20–42–67	7–26–19	65	Cancer.	N.	G.	7–24–19	At.	Operation by Dr. Cushing ? Spinal cord tumor. Died about January, 1920.
	10929	....	......	......	......	....	......	0 M 0–0–0–6 10 M 20–42–16–32	7–30–19	65						
125	12013	Pernicious anemia........	49	M.	—	U. S.	0	?	....	?	P. A. Tbc.	T.	G.	10–11–19	S.	Died, November 19, 1919.
126	11829	Pernicious anemia........	55	F.	—	U. S.	6	0 M 0–0 0 6 M 23 16	9–14–19	35	Neg.	T.	W.	9–13–19	T.	Died, December 13, 1919.
127	11999	Pernicious anemia. ? Sprue (recovered).	53	M.	—	U. S.	9	0 M 0–0–0 0 M 0–0–0	9–11–19	30	Neg.	T.	G.	9– 7–19	S.	Died, 12–8–19. P. M., Bone marrow, hyperplasia.
128	12208	Pernicious anemia........	56	F.	—	U. S.	3	0 M 0–0–0	11– 8–19	25	Neg.	T.	N.	11– 6–19	At.	No note.
129	12313	Pernicious anemia........	61	F.	—	U. S.	12	0 M 0–0 0 15 M 25 25–30	11–22–19	55	Cancer. Tbc.	T.	G.	11–20–19	T.	Died, 12–4–19. P. M., Pernicious anemia.

* Upper figure is the free HCl, lower is the total acid, expressed in c. c. of N-10 HCl per 100 c. c. stomach contents M indicates test meal.
† Hemoglobin determinations made the same day as gastric analysis or within a few days. ‡ T = typical. S = suggestive. N = normal.
§ G = gray. W = white. N = normal. ‖ T = typical. S = suggestive. At = atypical.

TABLE 1—CONTINUED

Serial number	Hospital number	Clinical diagnosis	Age	Sex	Wassermann	Birthplace	Eosinophilia, per cent	Gastric analysis *	Date of gastric analysis	Hemoglobin, per cent †	Family history	Tongue ‡	Hair §	Date of admission	Blood smear ‖	Follow-up notes
130	12322	Pernicious anemia. Vitiligo.	56	F.	—	Canada.	5	0 M 0-0	11-23-19	55	Cancer. Tbc.	S.	G.	11-21-19	T.	Doing pretty well. 8-30-20.
131	11710	Pernicious anemia........	42	M.	—	U. S.	11	0 M 0-0-0	?	?	Neg.	T.	N.	9-18-19	S.	No note.
132	13032	Pernicious anemia........	50	F.	—	U. S.	4	0 M 0-0-0 10 M 2-5-9	2- 4-20	85	Tbc.	T.	G.	2-23-20	S.	No note.
133	12607	Pernicious anemia. Arterio sclerosis.	59	M.	—	U. S.	6	0 M 0-0-0 8 M 17-30-20	1-29-19	45	Cancer.	T.	G.	12-28-19	S.	No note.
134	12633	Pernicious anemia. Ataxic paraplegia. Hypertension.	42	M.	—	U. S.	0	0 M 0-0-0 2 M 7-11-12	1- 7-20	100	Neg.	N.	G.	1- 1-20	S.	No note.
135	12699	Pernicious anemia. Ataxic paraplegia. Arterio-sclerosis.	55	F.	—	Canada.	6	0 M 0-0-0	1-17-20	84	Neg.	S.	G.	1-12-20	S.	No note.
136	12725	Pernicious anemia........	67	F.	—	U. S.	1	0 M 0-0	1-17-20	35	Neg.	S.	W.	1-15-20	T.	No note.
137	12235	Pernicious anemia. Chronic myocarditis. Acute pulmonary œdema.	55	F.	—	Canada.	1	?	....	?	Neg.	T.	?	10-10-19	T.	No note.
138	13167	? Pernicious anemia. Cardiac hypertrophy.	41	F.	—	Ireland.	2	0 M 0-0-0 37 M 10-16-3	3-16-20	60	Neg.	N.	G.	3-15-20	S.	No note.
139	13226	Pernicious anemia. Diabetes mellitus.	50	F.	—	Denmark.	5	?	....	?	Neg.	N.	G.	3-24-20	T.	No note.
140	13266	Pernicious anemia. ? Mitral stenosis and mitral insufficiency.	40	F.	—	Canada.	1	?	....	?	Tbc.	N.	G.	3-20-20	T.	Transfused in 1917 for anemia. September 1, 1920, not doing well.
141	13635	Pernicious anemia........	52	F.	+	Canada.	0	0 M 0-0-0 2 M 10-10-8	5-26-20	44	Cancer. Tbc.	T.	G.	5-24-20	S.	No note.
142	13262	Pernicious anemia. Chronic bronchitis. ? Syphilis.	69	F.	+±	U. S.	2	0 M 0-0-0 4 M 3 3-4	4-10-20	55	Neg.	N.	G.	3-29-20	S.	No note.
143	13783	? Pernicious anemia......	59	F.	—	Armenia.	4	0 M 0 0 0 3 M 3-12-11	6-16-20	31	Neg.	N.	G.	6-15-20	S.	Atypical: had great deal of diarrhea. ? Pellagra or sprue or cancer.
144	294	Pernicious anemia........	37	M.	—	Canada.	0	0 M 28 16 M 68	7-21-16	74	?	T.	N.	8-23-13	S.	Sept., 1920. Well and working.
145	3709	Dibothriocephalus latus..	33	F.	—	Sweden.	8	0 M 0 12 M 6	12- 2-15	40	Neg.	T.	N.	11-30-15	T.	Four worms delivered. Typical pernicious anemia picture.
								0 M 0-0 9 M 12-5	8- 9-16	124	....	......	......	....	......	May, 1918. Well.
146	3729	? Pernicious anemia. ? Anemia secondary to gasoline fumes.	17	M.	—	U. S.	20	0 M 0-0-0 8 M 36-16-6	12- 8-15	37	Neg.	T.	N.	12- 4-15	T.	
								0 M 0-0-0 0 M 5-14 14	1-15-16	83						
								0 M 0-0-0 0 M 16-23-5	2- 4-16	126	....	......	......	....	......	September, 1920. Well ever since discharge.
								0 M 0-12 8 M 18-43	10- 8-16	118						
147	6225	Chronic nephritis. Chronic uremia. Pernicious anemia.	62	F.	—	U. S.	..	0 M 0-0-8 0 M 28-22-32	3- 1-17	41	Neg.	N.	G.	2-28-17	S.	Atypical case. White count 46,000. Phthalein 0%. Died, 3-19-17. P. M., acute pericarditis, pleuritis, peritonitis and enteritis; chronic cholecystitis and peritonitis; chronic vascular nephritis, but nothing suggestive of pernicious anemia.
148	6375	Pulmonary tuberculosis. Renal tuberculosis. ? Pernicious anemia. Stomatitis.	45	F.	—	Canada.	4	0 M 0-?-0 10 M 0- 20	4- 6-17	78	Cancer.	S.	N.	4- 2-17	S.	Died, March, 1918. P. M., tuberculosis of lung, intestines and kidney; sprue, but no pernicious anemia.
	7297	Sprue, pulmonary tuberculosis. Secondary anemia.														
149	6872	? Cancer of cæcum. ? Pernicious anemia.	42	F.	—	U. S.	1	0 M 0-0-0	7- 3-17	54	Neg.	N.	N.	7- 2-17	At.	Operated on August 5, 1918. Cancer found. Died, March, 1919.
	7105	....	......	......	......	....	......	0 M 23-0-0 15 M 31-8-6	8-22-17	55	....	......	......	8-20-17		
150	7609	Syphilis. Syphilitic neuritis.	64	M.	++	England.	0	?	....	?	Cancer.	N.	G.	11-22-17	At.	
	8772	? Pernicious anemia. Syphilis.	......	......	++	....	9	0 M 2-6 16 M 20-20	6- 9-18	60	....	......	......	6- 5-18	T.	Died, January, 1919.
	9166	? Pernicious anemia. ? Hodgkin's disease. Syphilis.	......	......	......	....	......	....	....	......	....	......	......	8-26-18		

* Upper figure is the free HCl, lower is the total acid, expressed in c. c. of N-10 HCl per 100 c. c. stomach contents. M indicates test meal.
† Hemoglobin determinations made the same day as gastric analysis or within a few days. ‡ T = typical. S = suggestive. N = normal.
§ G = gray. W = white. N = normal. ‖ T = typical. S = suggestive. At = atypical.

Omitting these two last cases in which the diagnosis was questionable, in only one case in the 105, or 0.95 per cent, was the presence of free HCl demonstrated.

Three patients under observation showed a gastric anacidity, with a normal blood; but later all developed the typical picture of pernicious anemia. In one patient (Case 66) anacidity had been found six years before the diagnosis of pernicious anemia was made. At that time the hemoglobin was 90 per cent, and the blood smear normal. In the second patient (Case 73) a diagnosis of achylia gastrica and gastric hypermotility with normal blood, was made twenty months before the pernicious anemia became evident. In the third (Case 5) arterio-sclerosis, secondary anemia and achylia gastrica were diagnosed, three months before the pernicious anemia could be recognized. These observations indicate that the changes in the gastric contents antedate those in the blood and, together with the analyses presented, show that the anacidity is persistent here throughout the course of the pernicious anemia irrespective of the improvement in other findings so characteristic of this disease.

Of special importance is the group of cases in which the gastric analysis was essentially the determining factor which made us hesitate to make a diagnosis of pernicious anemia, and which suggested that we might be dealing with some other disease. As will be shown by a discussion of these cases, the presence of free hydrochloric acid in the gastric secretion throws doubt on the diagnosis even when all other factors point to pernicious anemia.

Special Cases

The following seven cases are included in Table I and are discussed here in detail because, although they suggested pernicious anemia in many respects, subsequent developments proved that some other condition was present and that the anemia was of a secondary type. Five of these cases demonstrate the importance of questioning the diagnosis of pernicious anemia when free hydrochloric acid is present in the gastric juice.

Case 144.—A painter, 37 years of age, was admitted August, 1913. Complaint: shortness of breath and an infected hand. The family and past history were negative. He had been subject to attacks of abdominal pain of a few days' duration, but not of sufficient severity to force him to stop work. Slight jaundice developed during these attacks. Two weeks before admission he had cut the thumb of his left hand. The wound had become infected and it was for this reason that he entered the hospital. His shortness of breath never had been very troublesome. Physical examination showed a marked brownish pigmentation of the skin, no lead line, a peculiar yellowish appearance in the scleræ, more like fat deposits than jaundice, and a markedly enlarged spleen, 10 cm. below the left costal margin. The patient had noticed that his skin had been growing darker during the past year. The urine never contained bile. The blood showed a hemoglobin of 65 per cent; erythrocytes, 2,544,000; leucocytes, 7,500. Differential: polymorphonuclears, 67 per cent; large lymphocytes, 27.5 per cent; small lymphocytes, 3.5 per cent; eosinophiles, 1.5 per cent; basophiles, 0.5 per cent. In the smear were noted marked poikilocytosis and anisocytosis, slight diffuse polychromatophilia and stippling and an occasional nucleated red cell. The color index was persistently above 1. The patient had no fever and no gastric analysis was made at this time. He was thought to have pernicious anemia. He was discharged in good health and has remained so.

In July, 1916, he was called back to the hospital. He seemed to be in the same general condition as when seen three years before. He had had no new symptoms and was working hard. At this time he had a recrudescence of a chronic gonorrhea. The hemoglobin was 74 per cent, the erythrocytes were 4,400,000, and the smear showed a slight variation in the size and shape of the erythrocytes, predominating microcytes with occasional macrocytes. The tongue was smooth and pale and the skin was markedly pigmented. No bile was present in the urine. The liver was not felt but the spleen was more than a hand's breadth below the left costal margin. Gastric analysis showed a normal quantity of free hydrochloric acid. A fragility test of the red cells was made and hemolysis was found to begin at 0.43 per cent NaCl and to be complete at 0.33 per cent; a normal control done at the same time giving 0.36 per cent and 0.25 per cent. There was, therefore, a slight increase in the fragility of the erythrocytes. To-day the patient is working and feeling well. The most probable diagnosis made by two observers was hemolytic jaundice, for certainly it would have been very unusual for a case of pernicious anemia to go for seven years with no symptoms of the disease other than the suggestive blood picture.

Case 145.—A Swedish woman, 33 years of age, entered the hospital with a history of two attacks similar to her present complaint, one eight years and the other two years before admission. She had enjoyed her customary good health between these attacks. The present upset had begun two months before, and was characterized by vomiting, weakness and increasing pallor. Physical examination showed pallor, increased pigmentation of the skin, and a systolic murmur at the base of the heart. The blood examination was typical of a primary anemia—a leucopenia, high color index, nucleated red cells and variation in size and shape of the erythrocytes. The Wassermann test was negative. She had some fever and the heart rate was accelerated. The gastric analysis showed complete anacidity. The presence of fish tapeworm eggs was discovered, and, as a result of treatment, four complete dibothriocephalus latus parasites were passed. The patient made a complete recovery both symptomatically and as far as her blood picture was concerned, and remained perfectly well for the two years during which we were in touch with her. When seen ten months after her admission to the hospital, the hemoglobin was 124 per cent (Sahli), but the gastric juice still showed anacidity.

Case 146.—A boy, 17 years of age, entered the hospital December 4, 1915, stating that eight weeks previously his parents noticed that he was growing pale. Two weeks later he began to feel weak, lost his appetite and was often nauseated and vomited. Three weeks before admission he stopped work on account of weakness, dyspnea, dizziness and headache. He did not have any pain, but often felt cold and chilly. Physical examination showed marked pallor, a slightly enlarged heart with a loud systolic murmur over the precordium. The edge of the spleen was felt 2 cm. below the left costal margin. The blood picture was typical of pernicious anemia. The hemoglobin was 37 per cent; erythrocytes, 1,304,000; leucocytes, 6,400, and the stained smear showed nucleated cells, macrocytes, microcytes, granular and diffuse polychromatophilia. Three gastric analyses done during his stay in the hospital all showed complete anacidity. The Wassermann test and blood cultures were negative. He ran a fever over 100° most of the time with a heart rate of about 100. He gradually improved without medication and, when discharged, the blood and physical examinations were normal. As a mechanic's helper in a garage, the patient had been exposed to exhaust fumes, and although most of the important features of the case pointed to the diagnosis of primary anemia, this was doubtful, and he was discharged with a diagnosis of questionable pernicious anemia, or anemia secondary to occupation. This patient, when seen ten months later, seemed to be in perfect health. He had changed his occupation. His hemoglobin then was 119 per cent and he had 6,136,000 erythrocytes per cubic millimeter. At this time the gastric analysis did not show a persistent anacidity, for in the second sample after the test breakfast the free hydrochloric was 12. He has remained well for five years and has been working all the time. In the light of the progress of this case, pernicious anemia can be safely ruled out, and it is interesting to note that this patient in whose case all the findings pointed to pernicious anemia should have begun, on recovery, to show free hydrochloric acid in the gastric juice, in contrast with patients suffering from a true pernicious anemia.

Case 147.—A married woman, 62 years of age, entered the hospital February 28, 1917. For one year she had been complaining of nausea and gas in the stomach, not particularly related to meals or effort. These symptoms had steadily grown more pronounced and she had lost some weight and strength. She had never had any pain or vomiting. Physical examination showed marked pallor of the skin and mucous membranes, with a slight suggestion of a lemon tint. The tongue was not smooth. There was very little else of a definite nature made out except for a suspicious mass in the center of the abdomen. The x-ray examination of the gastro-intestinal tract was negative except for a low position of the stomach. The blood examination showed: hemoglobin, 40 per cent; erythrocytes, 1,730,000; leucocytes, 4,300. Differential: polymorphonuclears, 74 per cent; large lymphocytes, 23 per cent; small lymphocytes, 1 per cent; eosinophiles, 2 per cent; basophiles, 0. There was slight variation in the

size and shape of the erythrocytes, but otherwise nothing remarkable. A phenolsulphonephthalein test, done twice, showed 0 per cent in two hours. Gastric analysis showed an absence of free hydrochloric in all specimens except the last in which it was 8. After being in the hospital for twelve days, she had a convulsion, followed later by periods of delirium. She improved, but then developed signs of an acute pericarditis. She finally died with signs of either compression or consolidation of the left lower lobe of the lung. Clinically, the case was considered to be one of chronic nephritis and uremia, chronic peritonitis, acute pericarditis and possibly pernicious anemia. The autopsy failed to show any evidence of pernicious anemia. There was acute pericarditis, pleuritis and peritonitis, chronic vascular nephritis and chronic cholecystitis. This case is reported in detail because the presence of free HCl in the gastric juice was enough to throw a doubt on the diagnosis of pernicious anemia, although the blood findings and general appearance of the patient were strongly in favor of that diagnosis.

Case 148.—A school teacher, 45 years of age, entered the hospital complaining of general weakness, sore mouth, and vaginal pain. For a period of about seven or eight years, up to five years before admission, she had had symptoms of tuberculosis, cough, bloody sputum, night-sweats and fever. While in Mexico for her health five years before, she had had symptoms similar to her present illness. For three months she was greatly troubled with pain and burning of her tongue. She also had similar sensations in the vagina. She lost strength. Her condition repeatedly improved for a few days and then she relapsed. Physical examination showed a reddened, tender tongue and small hæmorrhagic punctate areas on the mucous membrane of the mouth. There were also signs of consolidation at the apex of the left lung. The general physical examination was not otherwise particularly significant. The urinary findings suggested the possibility of renal tuberculosis. Gastric analysis showed a persistent absence of free HCl in all specimens. The blood gave: hemoglobin, 78 per cent; erythrocytes, 2,748,000; leucocytes, 7,500. Differential: polymorphonuclears, 63 per cent; large lymphocytes, 32 per cent; small lymphocytes, 2 per cent; eosinophiles, 2 per cent; basophiles, 1 per cent. There were many macrocytes and microcytes, but no nucleated cells. The temperature rose in the evening to just above 99° most of the time and the pulse was around 80. At this time, although sprue was considered, the condition was regarded as pulmonary tuberculosis, possible renal tuberculosis and a possible pernicious anemia. She returned to the hospital five months later, complaining of cramp-like pains in the abdomen and diarrhœa with blood and mucus in the stools. It was now thought that she had sprue in addition to the pulmonary tuberculosis. She left the hospital improved, but died about six months later. An autopsy showed tuberculosis of the lungs, left kidney, bladder, and large intestine. (Dr. A. W. Sellards thought that a diagnosis of sprue should be added.) This case is included as one in which there were many features pointing to pernicious anemia but in which generalized tuberculosis or sprue was the underlying factor. Recently Wood[3] has called our attention to the frequency of blood changes in sprue similar to those of pernicious anemia. Possibly in some of these patients the gastric content will show the presence of free HCl, and then pernicious anemia can be ruled out, but in the case just cited there was complete anacidity.

Case 149.—A trained nurse, 42 years of age, complained of increasing weakness, dyspnea and palpitation of the heart. Twelve years previously she had had an attack of acute pain in the right lower quadrant, diagnosed as acute appendicitis. Three months before admission there had been a recurrence of acute pain in the right lower quadrant and on three occasions bloody stools had been passed. Physical examination showed a well-nourished, middle-aged woman with marked anemia and a lemon tint to the skin. A loud systolic murmur could be heard over the precordium, and a large mass was felt in the region of the cæcum. The tongue was not smooth. The blood showed: hemoglobin, 54 per cent; erythrocytes, 3,528,000; leucocytes, 7,500. In the stained smear were noted a moderate amount of anisocytosis and poikilocytosis, considerable achromia, and two nucleated red cells. The gastric analysis showed an absence of free HCl in the fasting content, but a free HCl of 23 in the first sample after the test breakfast. Although the x-ray of the gastro-intestinal tract was negative, the case was diagnosed: pernicious anemia (?) and carcinoma of the cæcum (?). She was discharged unimproved, and it was learned that a year later a malignant tumor of the abdomen was discovered at operation and she subsequently died. Here, again, we have a case that suggested pernicious anemia in some respects, but which showed free HCl in the gastric contents. Later on it was proved that the anemia was due to carcinoma of the cæcum.

Case 150.—A Hebrew, 67 years of age, entered the hospital complaining of pain in the left hip of three months' duration. His past history and family history were unimportant. His present trouble had begun with pain in the hip extending down to the ankle, and difficulty in lifting his foot. Some days later pain had appeared in the left shoulder and elbow. These pains had kept on recurring and growing more intense so that he had had to stop work and could not sleep; they had become shooting in character and extended to both arms and legs. He had had fever and severe chills and had lost some weight. Physical examination showed peripheral arteriosclerosis. The heart was normal except for a loud systolic murmur all over the precordium. The inguinal glands were enlarged and an old scar was present on the penis. There was a definite Romberg's sign with increased deep reflexes of the legs. No Babinski or clonus. There was considerable tenderness on pressure over the nerve trunks of the legs and arms. The extensor surfaces of the arms and hands were abnormally pigmented. The Wassermann reaction was double plus. The patient ran an irregular fever, 100-102°, with a pulse rate between 70 and 90. He was in the hospital on three different occasions, improving each time, first under anti-luetic treatment and later after transfusions. The blood showed: hemoglobin, 60 per cent; erythrocytes, 2,776,000; leucocytes, 11,900. Differential: polymorphonuclears, 73 per cent; lymphocytes, 9 per cent; transitionals, 14 per cent; eosinophiles, 4 per cent. There was variation in size and shape of the erythrocytes, some macrocytes, and an occasional nucleated red corpuscle. The diagnosis was not certain, but the case was considered as one of pernicious anemia and syphilis. A gastric analysis showed a slight amount of free HCl in the first two samples of the test breakfast, the figures being 2 and 6. We considered this to be sufficient to throw considerable doubt on the diagnosis of pernicious anemia. Subsequently, an inguinal gland was excised and the pathologist reported a lymphoblastoma of the Hodgkin's type. The patient died about 14 months after his first admission to the hospital.

The above seven cases gave evidence in the general history, physical examination, or the blood findings, suggesting a more or less positive diagnosis of pernicious anemia, although the final outcome made it unlikely that pernicious anemia had anything to do with the condition. One of these seven patients turned out to have a dibothriocephalus latus infection and was apparently cured after this had been treated. She had absolute anacidity on two different occasions. Another was found to have sprue and tuberculosis of the lungs, kidney, bladder and intestines. This patient also showed gastric anacidity on two different occasions. In all the other five the stomach contents showed some free HCl. One was probably a case of hemolytic jaundice, a second was an atypical type of secondary anemia resulting from toxic fumes to which the patient had been exposed in his work; the third was a case of chronic nephritis and cholecystitis with terminal acute pericarditis, pleuritis and peritonitis; the fourth was cancer

of the cæcum, and the last was Hodgkin's disease and syphilis. We feel from the evidence in this group of cases that if any free hydrochloric acid is found in the gastric contents one should seriously question the diagnosis of pernicious anemia. Conversely, absence of free hydrochloric acid suggests the possibility of pernicious anemia and other features of the disease should be carefully sought.

The Incidence of a Family History of Anemia

Bartlett[4] gives an account of a family of eight in Vermont, father, mother, five sons and one daughter. The father died at 44 of anemia with symptoms of pernicious anemia. There was no blood examination, and the autopsy failed to show the cause of death. The mother died at 60 of anemia, but there was no autopsy or blood examination. One son died at 24 of anemia with symptoms of pernicious anemia. There was no blood examination but autopsy showed findings typical of pernicious anemia. A second and third son died at 35 of typical primary anemia, confirmed by autopsy and blood examinations. A daughter was alive at 49, with a marked chlorosis, having a hemoglobin of 40 per cent and 5,000,000 red cells. All these people lived on the same farm and the only two sons who did not have anemia lived elsewhere most of the time.

With the above facts in mind the family history of all our cases of pernicious anemia has been carefully studied, and appreciating that cases of pernicious anemia, when incorrectly diagnosed, are apt to be ascribed either to cancer or tuberculosis, the incidence of these two diseases as well as that of anemia was determined. Of the 143 cases, in 41 there was a definite family history of cancer or tuberculosis or both. (In the family history are included sisters and brothers, the parents and children.) In nine cases it was possible to obtain a definite history of pernicious anemia in other members of the family, of whom six have died. In two there was a history of members of the family dying of anemia, but it was not known whether the anemia was of the pernicious type or not, and in 91 cases the history was negative. There was a frequent familial occurrence of cancer and tuberculosis among these patients. Probably some cases of true primary anemia are included because of the difficulty in differential diagnosis in general practice. Considering that 6.3 per cent of these patients have a positive family history of pernicious anemia, we are impressed by the fact that there is an important familial factor in this disease, but whether this factor is one of heredity or one of environment our analysis does not reveal.

Racial Discrepancies in the Prevalence of Pernicious Anemia

Upon this point there are differences in opinion and since there is not sufficient evidence as yet to support definite conclusions, an analysis of the present series of cases may be to the point.

Cabot[5] in his exhaustive review of pernicious anemia, in discussing etiological factors, states: ". . . . Race, civil condition, residence, do not seem to have any special bearing."

TABLE 2*

	All countries	Born in U. S.	Total foreigners	Russia	Canada	Ireland	England	Italy	Sweden	Denmark	Germany	Greece	Miscellaneous	All foreign countries except Ireland, England, Sweden and Denmark
Total admissions to hospital.	23323	13696	9627	2193	2070	1813	795	668	278	37	358	323	1092	4634
Per cent admissions to hospital.	100	58.7	41.3	9.4	8.9	7.8	3.4	2.9	1.2	0.2	1.5	1.4	4.7	19.9
Total P. A. cases.........	143	86	57	3	23	11	8	1	6	2	1	0	2	7
Per cent P. A. cases......	100	60.1	39.9	2.1	16.1	7.7	5.6	0.7	4.2	1.4	0.7	0	1.4	4.9
Per cent of all admissions born in various countries having P. A.	0.61	0.63	0.59	0.14	1.11	0.61	1.01	0.15	2.16	5.4	0.28	0	0.18	0.15

* These figures include all admissions to hospital to January 1, 1921.

Silberman[10] made an extensive study of the occurrence of pernicious anemia in East Prussia and concludes that locality has something to do with the occurrence of the disease among certain races. Although statistically definite conclusions from the figures here given are not warranted, the following suggestive figures are interesting:

We have used two ways of determining the influence of such factors in this series: First, we have compared the percentage of the total number of admissions to the hospital from a given country with the percentage of the total number of pernicious anemia cases in persons born in that country (see 2d and 4th lines of Table 2). Thus, 9.4 per cent of all medical admissions came from Russia, but only 2.1 per cent of the cases of pernicious anemia are Russian immigrants. Contrast the Canadians—whereas 8.9 per cent of all admissions came from Canada, yet 16.1 per cent of the pernicious anemia cases were in Canadians. In these two countries the total number of admissions is sufficiently large to give these statistics some value. There were 2193 Russians and 2070 Canadians admitted, but only 3 Russians with pernicious anemia as compared with 23 Canadians. We feel that it is not possible to ascribe

this striking difference merely to chance. A similar discrepancy arises in comparing Sweden and Italy. There were two and one-half times as many Italians as Swedes admitted, but there was only one Italian with pernicious anemia, whereas there were six Swedes.

This will be made clearer by reference to the last column of Table 2, in which admissions from foreign countries are tabulated, excluding those in which the disease seems to be more prevalent, i. e., Canada, Ireland, England, Sweden and Denmark. There were 4634 admissions or 19.9 per cent of our cases from the foreign countries not so excluded, yet there were only 7 or 4.9 per cent cases of pernicious anemia from these other countries. This suggests that the disease is about one-fourth as prevalent among Eastern Europeans as among citizens of the United States and the five foreign countries mentioned above.

The second basis of comparison is the ratio of the number of patients with pernicious anemia born in a given country to the total number of admissions to the hospital for all causes from that country (Table 2, line 5). For example, 0.61 per cent of all patients admitted to the hospital have pernicious anemia, but over 1 per cent of all patients from Canada and England and over 2 per cent of those from Sweden had the disease, in contrast to only 0.14 per cent and 0.15 per cent of those from Russia and Italy respectively. The foreign countries charted in the last column of Table 2 show a low incidence of the disease, only 0.15 per cent, or about one fourth the general admission rate. Inasmuch as almost all our Jewish patients of middle or advanced age have been born in Russia, and we have a fairly large percentage of Jewish admissions, the infrequency of the disease in Russian-born patients bears out the clinical impression that the disease is comparatively infrequent in the Jewish race. Similar tables from other hospitals accumulating a large statistical basis will be necessary before the evidence for these racial discrepancies is conclusive, but it is certainly suggestive from this small group that pernicious anemia is more frequent in Americans and immigrants from Canada, England, Ireland, Sweden and Denmark than in those from Russia and Italy and eastern Europe.

Syphilis and Pernicious Anemia

It is known that syphilis may occasionally produce a rather severe degree of anemia which may sometimes present a blood picture that can readily be compared with that of pernicious anemia. With this in mind, an analysis was made of the incidence of a positive Wassermann reaction in the blood of all the cases studied. In two of the 143 cases a Wassermann test was not made and of the remaining 141, all were negative except six, *i. e.*, 4.3 per cent were positive. Inasmuch as the average incidence of a positive Wassermann reaction in all medical admissions to this hospital is about 12 per cent [6] we can readily see that the incidence among these cases is one third of the average. Of these six positive cases, there was a definite past history of a hard chancre in but one. Three of the patients died within 29, 16 and 12 months, respectively, after their admission, despite intravenous treatment for syphilis. Each presented the findings typical of pernicious anemia in the blood and gastric contents and on general physical examination. Of the other three patients who gave positive Wassermann reactions one has done quite well for four and one-half years after receiving three intravenous injections of salvarsan. Another has been doing pretty well for three years following three intravenous treatments with salvarsan, and the last one left the hospital six months ago, after receiving a similar course of treatment. These patients also gave quite a typical clinical picture of a primary anemia.

From the above data the conclusion is warranted that in our cases, syphilis has borne very little relation to the development of pernicious anemia. Furthermore, when the blood of a patient presenting findings of pernicious anemia has given a positive Wassermann reaction, the course of the disease has not been changed and antisyphilitic treatment has had no effect upon it. (The two patients who have done well for four years and seven months and three years, respectively, will have to be followed for a much longer time before they can be used as evidence against this statement.)

The Frequent Occurrence of Eosinophilia

In a study of 110 cases by Cabot [7] it was found that an eosinophilia of over 4 per cent was found in 13 per cent of the cases on the first blood examination. The occasional occurrence of marked eosinophilia has also been observed in pernicious anemia. Beifeld and Barnes [8] reported a case of severe anemia with eosinophilia ranging between 9 per cent and 53 per cent. The case was fairly typical of pernicious anemia in other respects, but the high eosinophilia led them to consider this as a remote probability.

In the cases here reported frequent blood examinations were made and the occurrence of more than the usual percentage of eosinophilic leucocytes was very striking. It must be borne in mind that the following figures include some patients who on one or two occasions only showed the increase in these cells, although many had a more persistent eosinophilia. Of the 143 patients studied (see Table 3), 89 never showed more than 4 per cent of eosinophiles; of the 43 patients who showed 5-9 per cent eosinophiles, 12 had 5 per cent, five had from 10-14 per cent, four from 15-19 per cent, one from 20-24 per cent and one had 28 per cent of eosinophiles. In other words, 54 patients (37.8 per cent) had 5 per cent or more of eosinophiles and 29.4 per cent had 6 per cent to 28 per cent of eosinophiles.

TABLE 3

Eosinophiles	0–4%	5–9%	10–14%	15–19%	20–24%	25–30%	Total
No. of cases	89	43	5	4	1	1	143
Per cent	62.2	30.1	3.5	2.8	0.7	0.7	100

The significance of the above figures is that a high eosinophile count need not militate against the diagnosis of perni-

cious anemia, although the stools show no evidence of parasites. In one patient with the typical clinical and blood picture of pernicious anemia, dibothriocephalus latus eggs were found and four complete worms were expelled, but her blood showed only 8 per cent eosinophiles.

"The Typical Blood Picture"

An attempt was made to determine the frequency of typical blood findings in the disease, taking into consideration the presence of macrocytes and deeply stained red cells, the high color index, the low white blood cell count, the appearance of nucleated erythrocytes, and the variation in the size, shape and staining qualities of the red blood cells. The decision as to whether a blood finding is typical, suggestive or atypical of pernicious anemia is an arbitrary one and of course might well differ in the opinion of different observers. However, at a hospital where considerable interest is manifested in blood diseases and where a fairly large number of cases of anemia are seen, the general impression does not differ much from the average opinion in other clinics. Of the 143 cases, the blood examination was considered typical in 110, or 76.9 per cent, suggestive in 26 or 18.2 per cent and atypical or within normal limits in 7, or 4.9 per cent. The fact that about 23 per cent did not have the blood typical of the disease is of importance in relation to the much rarer findings of free hydrochloric acid discussed above. In fact a blood picture typical of pernicious anemia may be present in other diseases and in such cases the gastric analysis becomes an important help in making the proper diagnosis.

Unnecessary Abdominal Operations in Pernicious Anemia

It is not infrequently found that patients suffering from pernicious anemia have been operated on previously for the same complaints that eventually bring them to a medical clinic. There were six such patients out of 125 in this series

TABLE 4

Case number. See Table 1	Operation	Interval between operation and admission	Result
Case 1....	Gastric ulcer..........	2 years...	None found.
Case 30....	Gastric ulcer..........	11 years...	None found.
Case 79....	Gastric ulcer..........	4 months.	None found.
Case 103....	Gastric cancer	19 months.	None found.
Case 136....	Gall-stones	4 years...	None found.
Case 94....	Chronic cholecystitis...	6 years...	Symptoms persisted.

who were definitely questioned concerning any previous operation. Three had been operated upon for gastric ulcer and one for gastric cancer, but no such lesion had been found. Two had been operated upon for gall-bladder disease, but the same symptoms had persisted after operation. In these six cases operative procedures had been fruitless, although it is quite well known that patients with pernicious anemia occasionally come to autopsy showing either gall-stones or chronic cholecystitis. However, it is very unlikely that the abdominal symptoms of pernicious anemia are relieved by surgical intervention and some of these operations might have been prevented by more careful medical study of the case.

The Appearance of the Tongue

The appearance of the tongue is generally considered to be of distinct aid in diagnosis. "Typically" the tongue is smooth, glistening and pale, the papillæ are atrophic, especially around the edges. Wallgren[9] has carefully studied these tongue changes. With these criteria the appearance was described as typical of pernicious anemia, suggestive, or within the normal limits. Of the 143 cases, notes on the tongue, made in 127, describe it in 82, or 64.4 per cent, as typical; in 24, or 18.9 per cent, as suggestive, and in 21, or 16.5 per cent, as within normal limits. In more than half the patients, therefore, the tongue is typical in appearance. The simplicity of the observation gives it great value in suggesting the disease and in selecting those patients who should have more careful study.

The Occurrence of Prematurely Gray Hair

In observing large numbers of patients suffering from pernicious anemia, the clinician is impressed with the great prevalence of gray hair. In fact, he is often struck by its beautiful white, silky appearance. Of course, it is evident that a large number of adults might well have gray hair, and as the average age incidence of this disease is about fifty years, it is not surprising that the hair is frequently found to be gray or white. Notwithstanding this, however, it seems fair to say that patients with pernicious anemia are prone to have gray hair and even white hair prematurely; in fact, a fair number of the comparatively younger cases show it. A note as to the color of the hair was made in 119 of the 143 cases. Considering the cases under fifty years of age, we find the following:

Of 4 cases in the 3d decade, 50 per cent had gray or white hair
Of 12 cases in the 4th decade, 33 per cent had gray or white hair
Of 37 cases in the 5th decade, 78 per cent had gray or white hair

Sex Incidence

There is some difference of opinion as to whether pernicious anemia is more frequent in males or females. Cabot thought that the statistics, according to which females predominated, must have included cases of anemia associated with pregnancy and which were, therefore, not true primary anemias. Osler[10] stated that the disease was more common among males. Colquhoun[11] in discussing pernicious anemia in New Zealand states that in fifteen years, in the whole of New Zealand, 1078 persons were reported as having died from anemia, of whom 537 were males and 541 females.

In this series of 143 cases, there were 59 males and 84 females (Table 5). Cases of anemia associated with preg-

nancy were observed in the hospital, but these were not diagnosed as pernicious anemia. The proportion, therefore, in the sexes is about as two to three, with the females predominating.

Prognosis

In following these patients to determine the end results as far as was possible, it was learned that 97 had died, of whom 48 were males and 49 females. The average age of the 48 males who died was 51.6 years and that of the 49 females was 50.9 years. Thirteen were discharged from the hospital within six months of the time this study was closed and no information concerning their condition was obtained. Thirteen could not be traced and 21 were alive. Of these 21 living patients six have lived more than four years after their first admission to the hospital.

TABLE 5

	Number of cases	Number of males	Average age	Number of females	Average age *
All P. A. cases....	143	59	51.7	84	50.4
Those dead.......	97	48	51.6	49	50.9
Autopsies	18	10	45.6	8	54.8

* All ages are those obtained on the first admission to the hospital.

The average age of the entire group on their first admission was 50.8 years. The average age of the 59 males was 51.7 and that of the females was 50.4 years. In regard to the predominance of females mentioned above, it would seem that if cases of pregnancy were included in these statistics the average age of the females would be considerably lower, for there were 25 more females than males and these 25 cases, if in any way associated with pregnancy, would have diminished the age of this group to an appreciably lower level than 50 years.

It is important to bear in mind that, at least in New England, pernicious anemia is not a rare disease and that in a hospital service probably more cases are seen than in the average outside practice because of the obscurity of the condition and the difficulty in diagnosis. However, during the period covered by this series of cases, 105 cases of typhoid fever and 401 cases of lobar pneumonia as against 143 of pernicious anemia were observed. The general course of the disease is very similar to that of cancer, *i. e.*, the overwhelming majority of the patients die within a few years, while a small proportion have remissions of considerable duration, as much as four or more years.

Summary

Our study of 150 cases of pernicious anemia includes seven patients in whom the subsequent course of the disease either indicated that the diagnosis was wrong or threw considerable doubt on its correctness. The gastric secretion in the fasting contents and after an Ewald test breakfast was analyzed in 107 of the 143 cases of pernicious anemia. Analyses were made repeatedly on the same patient in many instances when the blood condition was low and when normal, and some analyses extended over many years. In only three cases was free HCl found at any time in the gastric secretion, and in two of these cases the diagnosis of pernicious anemia was questioned. These figures, then, show a persistent anacidity in 104 or 99 per cent of cases of pernicious anemia. Pepsin was tested for in a small number of cases and was always found lacking.

Evidence has been brought forward that the changes in the gastric secretion occur very early in the disease, often years before the blood picture develops, and that once established they are never altered by the remissions so characteristic of the disease. The presence of free HCl in a suspected case of pernicious anemia is important evidence against this diagnosis. In five such patients who showed varying amounts of free HCl in the gastric secretion, operation, post-mortem examination, or subsequent findings practically ruled out pernicious anemia. Its absence should suggest pernicious anemia as a probable diagnosis worthy of careful consideration.

A distinct familial incidence was discovered in studying these 143 cases. In nine patients there was a definite family history of pernicious anemia in some other member of the family. In two others there was a history of death from an unknown type of anemia. Forty-one had a family history of tuberculosis or cancer or of both, and as these diseases are easily confused with pernicious anemia, these figures may well include cases of anemia.

There are racial discrepancies in the incidence of this disease. The figures studied would indicate that pernicious anemia is less frequent in the Italians, Russian Jews, and immigrants from Eastern Europe, than in Americans, Canadians, or immigrants from Ireland, England, Sweden or Denmark.

Syphilis bore no relation to the development or course of pernicious anemia in this series. Of 143 patients, six, 4.3 per cent, gave a positive Wassermann, which is about one-third the percentage of positive Wassermann reactions in all medical patients admitted to the hospital. When the Wassermann reaction was positive, anti-luetic treatment did not alter the course of the anemia.

The presence of eosinophilia is a common finding in pernicious anemia. Out of the 143 patients, 54 showed 5 per cent or more at one time or another. Even a very high eosinophilia—25 per cent or more—is not incompatible with the disease.

In 76.9 per cent of the cases the blood smear might be called typical of pernicious anemia, in 18.2 per cent it was suggestive and in 4.9 per cent it was atypical or within normal limits. This is in striking contrast with the more constant finding of anacidity.

Six of our patients had undergone previous abdominal operations for the symptoms similar to those that brought them into the hospital. Three of the operations were for gastric ulcer, one for gastric cancer, one for gall-stones, and one for chronic cholecystitis.

It was found that of 127 patients in whom the appearance of the tongue was described, in 63.8 per cent it was typically

smooth and atrophic, in 19.7 per cent it was suggestive, and in 16.5 per cent its appearance was about normal.

Notwithstanding the fact that this disease belongs to adult age, the hair seems to turn gray prematurely and often takes on a strikingly silky-white appearance.

The proportion of males to females in this series was as two to three with the females predominating. An analysis of the ages of these patients shows that the type of anemia associated with pregnancy which may be confused with pernicious anemia has not been included in these figures. The average age of all patients, of the males and females taken separately, and of those that have died is approximately the same—about 51 years.

Conclusions

Persistent absence of free HCl is practically a constant finding in cases of pernicious anemia. In our series it was noted in 99 per cent of the cases. It is often present years before the blood shows any of the typical changes, and possibly always antedates them. It is, therefore, of considerable importance in diagnosis.

There seems to be a distinct familial factor in pernicious anemia.

The incidence of this disease in English speaking people and Scandinavians was greater than in immigrants from Russia, Italy and Eastern Europe.

Syphilis played no significant rôle in this series.

Eosinophilia, even of a very marked degree, was a frequent finding.

The proportion of males to females was as 2 to 3. The average age of both sexes was about 51 years.

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GLAND PUNCTURE AS A DIAGNOSTIC MEASURE

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My attention was first directed to the possibilities of gland puncture as a diagnostic measure through the study of an imported case of human trypanosomiasis in 1912.[1] The patient had contracted the infection in the Belgian Congo three and a half years earlier and, after a rather characteristic course, the progress of the disease was arrested by injections of atoxyl. He was warned that he was not cured and would certainly have a relapse unless treatment was continued, but being free from symptoms he came to this country and remained apparently well until five weeks before he came under our observation. When we saw him, symptoms had returned but physical examination was negative except for a palpable spleen and general glandular enlargement. Repeated examinations of his blood and spinal fluid, both direct and after various methods of concentration, were negative for trypanosomes, and inoculation of susceptible animals with blood and spinal fluid failed to produce infection. Aspiration of an enlarged cervical gland with a syringe and small needle, however, yielded one or two drops of fluid in which was found one motile trypanosome. This finding was confirmed by the removal of a gland which was used for intraperitoneal inoculations of six white rats; all developed trypanosomiasis from which they eventually died.

Diagnosis by gland puncture was not a new method in trypanosomiasis, but had been recommended by Grieg and Gray[2] in 1904, and used with success by various workers in the sleeping sickness regions of Africa. It has also been used for the recovery of the etiological agent in syphilis,[3] in bubonic plague,[4] in filariasis[5] and in East Coast fever of cattle.[6] Although no reference to the use of this procedure in Leishmaniasis can be cited, the report by Cochran[7] of finding Leishmania by examination of excised cervical glands indicates that the simpler method would probably be successful in this disease also. A similar prediction might be made concerning yaws and leprosy, diseases in which there is often glandular enlargement and in which the causative organisms have been demonstrated in the glands.[8] In 1907, White and Proscher[9] announced the discovery of spirochætes in juice aspirated from the lymph glands of (1) a patient with acute lymphatic leukemia, (2) a patient with Hodgkin's disease, and (3) a guinea-pig previously inoculated with gland material from a case of lymphosarcoma. These findings lack confirma-

tion and various competent observers to whom the specimens were submitted expressed the belief that the spirochæte-like bodies seen were actually reticulum fibrils.[10]

Although a valuable aid in diagnosis, gland puncture had thus far been used only for the recognition of various etiological agents of disease, bacteria, protozoa and filariæ. It seemed possible that the method might be applied to the study of other conditions by the recovery of cellular material for microscopic examination. A few attempts were made in 1917 but no considerable series of cases was secured until 1920-1921. Even yet the series is too small, particularly the number of cases in some of the diseases studied, to permit of sweeping conclusions as to the value of the method. The results have seemed sufficiently promising, however, to warrant a brief report.

Method

In selecting a gland for puncture three requirements are essential. (1) The gland must be of a size sufficient to permit aspiration; tiny shot-like glands are not suitable for this purpose. (2) The location of the gland should be such as to render firm fixation possible and (3) injury of important structures improbable. By observing these points it has been possible in each instance to secure material from the gland and to avoid trauma of surrounding organs.

For the puncture a 2 c. c. Record syringe is used, equipped with a 21-gauge needle, 5 cm. in length. The point of the needle has a short bevel and is sharpened each time just before sterilization. After boiling needle and syringe, sterile salt solution is drawn into the syringe and then carefully removed, the syringe being held vertically with the needle downward, and the plunger moved in and out several times to expel all excess of fluid.

The skin over the gland selected for puncture is painted with iodine followed by alcohol. The gland is then firmly fixed by the fingers of an assistant, care being taken to have the skin over the gland stretched tightly; when fixation is satisfactory, the area to be stuck is again painted with iodine.

Before performing the puncture the plunger is pushed as far in as it will go and the syringe is held perpendicular to the skin. As the needle enters the gland, the sensation imparted to the person holding the syringe,while difficult to describe, is characteristic and unmistakable. The needle is inserted well into the gland, approximately half way through it. The syringe and needle are then rotated once about their longitudinal axis and the plunger is drawn out to the 1 c. c. mark to produce suction. The needle is then slowly withdrawn, negative pressure being maintained throughout or even increased by drawing the plunger back somewhat further. When the needle is completely withdrawn, there is heard a rush of air into the syringe through the needle, but only rarely is anything to be observed in the barrel of the syringe, at most only a drop or two of milky fluid. At first glance the results seem quite disappointing and it may appear that no material whatever has been obtained. In no instance, however, have I failed to secure some material, although from very firm, fibrotic glands the amount obtained may be very scanty. Ordinarily one secures enough to make two or three fresh preparations for dark-field examination and from six to twelve dried films for staining.

After the puncture the syringe is held either at an angle of 45°, the point of the needle almost touching the surface of a clean glass cover-slip, or vertically above the cover-slip. The plunger of the syringe is then pushed in, at first slowly, or if this is not productive of results, it is withdrawn and pushed in more sharply; by this means there is expelled a tiny drop of fluid. It has been our custom to use the first two or three drops to make fresh preparations for dark-field examination and the subsequent drops for pulling cover-glass films as in blood work. Some of these films are stained with a Romanowsky stain (Wilson's modification), others by the Ziehl-Neelsen method for acid-fast organisms, and if spirochætes are found on dark-field examination, one or two films are stained in a manner designed to give permanent preparations of these organisms. Other and special stains such as the Ehrlich triacid or the oxidase stain are applied if they are indicated by the results obtained with the Romanowsky stain.

An effort has been made to consider these gland puncture preparations objectively, to secure whatever information they presented and to try to arrive at a conclusion independently of knowledge from other sources. The findings obtained by this procedure have been checked, whenever possible, by examination of direct smear preparations and stained sections from the excised gland. Of assistance also in the luetic cases has been the Wassermann reaction and the presence of obvious signs of lues, while in the other cases, a study of the blood, the physical examination of the patient and the clinical course of the disease have been of great help in checking up the results of gland puncture.

Results

Thus far there has been an opportunity to apply this method of diagnosis in cases of syphilis, tuberculosis, Hodgkin's disease, acute and chronic lymphoid leukemia, acute and chronic myeloid leukemia, simple adenitis and in one instance each of trypanosomiasis and of metastasis of malignant disease.

A positive diagnosis dependent upon the recovery of the etiological agent of the disease was possible in a number of the cases.

As was mentioned above, the finding of trypanosomes established the diagnosis in our patient with African sleeping sickness.

Actively motile treponemata were found in a goodly percentage of the luetic cases, particularly from punctures of the inguinal glands in the late primary or early secondary stage of the disease. The relatively high incidence of positive results is to be attributed largely to the selection of favorable cases, but in the main the findings agree closely with those reported by others.[8]

Acid-fast organisms, morphologically indistinguishable from tubercle bacilli, were occasionally found in material aspirated from glands clinically tuberculous and later shown to be tuberculous by histological examination. They were

found once in a caseous gland and twice in glands which were completely broken down inside but which felt firm owing to the distention of the gland capsule by the contained pus. When pus was obtained, the syringe was filled and the aspirated material treated with antiformin. From glands which were neither caseous nor broken down, tubercle bacilli were not recovered.

Streptococci and staphylococci have been demonstrated in stained preparations from aspirated glands, but in the particular instances in which they were seen, unfortunately no tubes were inoculated so I cannot report having grown these organisms from material obtained by gland puncture. Streptococci were seen in one instance of subacute adenitis and were subsequently grown from the excised gland. Clumps of staphylococci were seen in the pus aspirated from a broken down tuberculous gland, evidently a secondary infection.

The results obtained from the study of the cellular material aspirated from glands are perhaps more interesting.

In Hodgkin's disease it has been possible to make a definite diagnosis in all of the cases examined thus far. The presence of eosinophiles, large endothelial cells and the peculiar multinucleated giant cells—the so-called "Dorothy Reed cells"—gives a characteristic and usually unmistakable picture.

In the cases of leukemia the gland picture has, to a remarkable degree, reflected the blood picture; such immature forms as were to be seen in the blood were also found among the cells from the gland, and hence, little information was added by the study of the material obtained on puncture. It is possible, however, that if the blood were in an aleukemic stage and recognition of the disease consequently more difficult, puncture of a gland might yield information of considerable value in arriving at a diagnosis.

In simple adenitis the picture is one of lymphoid hyperplasia, as is evidenced by the presence of large numbers of lymphoid cells in various stages of maturity, plus the presence of polymorphonuclear neutrophile leucocytes, the number depending upon the acuteness of the process. As has been mentioned, microorganisms responsible for the condition may also be seen occasionally.

In only one instance was a gland punctured which proved to be the site of a metastasis of a malignant tumor. Clinically, the case resembled Hodgkin's disease, but the blood picture was not that usually seen in any stage of Hodgkin's disease and the enlarged cervical glands, which had been treated by X-rays for a year, were very tender. The material obtained on puncture was very rich in cells the majority of which were of enormous size, some with one, others with several nuclei but practically all showing a vacuolated protoplasm. There were also multinucleated giant cells of a type quite different from "Dorothy Reed cells." Ordinary lymphoid cells were very scanty. Sections of an excised gland showed that all of the essential features of the picture had been observed in the puncture preparations. The diagnosis remains uncertain. Somewhat similar tumors have been seen arising from the nasopharynx and occasionally from the adrenal. From the sections it is evident that the gland is involved by a malignant tumor assumed to be metastatic, although the site of the primary growth has not been discovered. Following treatment with radium the glands have decreased markedly in size and the tenderness has practically disappeared.

Comment

The shortcomings of the method are as follows:

(1) From a fibrotic gland very little cellular material may be recovered, sometimes not enough to enable one to make a diagnosis.

(2) The cellular picture obtained is necessarily that from a very limited area of the gland, quite possibly not the area in which characteristic changes have occurred. A tuberculous gland, for instance, may show involvement in only one pole and none near the site of the puncture.

(3) The architecture of the gland is not shown as a rule, although occasionally a small bit of the gland is removed intact in the course of the puncture. The relation of the cellular elements to the reticulum, the amount of connective tissue reaction, the question of the invasiveness of the process present in the gland, are points on which information is rarely obtained from gland puncture.

Although these three points serve to limit the value of the method as a diagnostic aid it should be pointed out that in the great majority of glands which I have punctured (1) there has been no difficulty in securing adequate material for satisfactory preparations; (2) the disease process has not been localized but general throughout the gland; (3) the cellular picture has been sufficient to establish the diagnosis without a knowledge of the architecture of the gland. Whether the puncture has yielded the necessary information or not, the possibility still exists of excision of the gland for histological study and this procedure is to be recommended in all cases, at least until one has learned to interpret the results obtained by puncture. The appearance of cells stained with a Romanowsky stain and examined with an oil-immersion lens is so different from the appearance presented by cells of the same kind in an ordinary section stained with hæmatoxylin and eosin, that it requires some practice to build up a mental picture of the normal gland, to distinguish the normal from the abnormal elements and to recognize the types of pathological cells which are present.

Two other drawbacks might be mentioned as possibilities, (a) the spread of infection by puncture of infected glands—although this has not been observed in the patients with infected glands in this series—and (b) the dissemination of tumor cells when the process in the gland is malignant. I have punctured only one gland which was the site of a neoplasm and in that instance the patient had been regarded clinically as having Hodgkin's disease, as was mentioned above. Prompt radiation reduced the size of the glands and no ill effect from the puncture has been apparent. If the process in the gland were suspected of being malignant, one might well hesitate about undertaking a puncture, although in many instances it might establish the diagnosis without materially increasing the gravity of the outlook for the patient.

The advantages of the method may be set forth briefly:

(1) Rapidity; not infrequently a definite diagnosis may be made in 10 or 15 minutes.

(2) Thin preparations like blood films are secured, suitable for the application of a blood stain or special stains for cells, bacteria or protozoa and permitting the use of an oil-immersion lens in their study.

(3) The procedure is practically painless to the patient—more so than an ordinary venipuncture—and leaves no scar.

(4) It does not interfere with subsequent excision and histological study of the gland.

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THE PRESENT VIEWS ON ANAPHYLAXIS*

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The problems suggested by anaphylaxis are in reality manifold. They are at least three in number. First, we have to unravel the causes of the shock, to detect the factors to which the anaphylactic disturbances are due; this is a problem of etiology. Next, we have to discover which organs are primarily involved in the shock, and how the primary troubles can eventually bring about secondary disturbances; this is a question of symptomatology. The third problem has to do with general pathology; what is the rôle played by anaphylactic phenomena in the course, symptoms, or peculiarities, of several diseases, such as serum sickness, asthma, tuberculosis with its so much discussed hypersensitiveness toward tuberculin, intoxications with special reference to drug idiosyncrasy, and so on? There is no need to say that I cannot, in the brief space of time at my disposal, consider all of these problems; I intend to devote special attention only to the first of them, the predominant importance of which lies in the fact that it must necessarily be solved before any correct answer to the others can be arrived at. For example, the question as to the true anaphylactic nature of tuberculin hypersensitiveness cannot be decided until anaphylaxis itself has been suitably and strictly defined, such a definition of typical anaphylaxis depending upon a complete knowledge of its symptomatology and particularly of its cause. As a matter of fact, this essential point has not as yet been satisfactorily settled. Writers do not agree completely as to the extent and limits of the field of anaphylaxis. Some of them think that the term anaphylaxis ought to be retained to designate exclusively the phenomena depending upon the interaction of an antigen and an antibody. Others, myself included, strongly incline to the belief that the actual anaphylactic shock, with all of its characteristic symptoms, though resulting readily and frequently from such a cause, can also be brought about by quite different factors. However apt they may be to develop the shock, antigen-antibody complexes, nevertheless, in certain cases might be dispensed with. Were such an assumption true, the concept of anaphylaxis would expand in a much broader field, in which probably many morbid phenomena, occurring in the course of several diseases and apparently not dependent upon the activity of an antibody, could be included. Such being the case, the classification proposed by certain writers, which distinguishes completely, for instance, between allergy and

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drug idiosyncrasy and real anaphylaxis, ought to be revised. Undoubtedly, anaphylaxis would include more numerous phenomena and consequently gain a much larger significance, were the intervention of an antibody, at least in certain cases, not necessary.

It follows from these considerations that, among all the problems connected with anaphylaxis, the one most important—since on its solution depends the very definition of anaphylaxis—is to determine the fundamental reaction giving rise to the typical disturbance. In reality, it appears as though many investigators had devoted themselves less to the understanding of this interaction in its intimate nature than to the determination of its localization. The point most vehemently in dispute at the present time is that of the site of the reaction which is the immediate causal factor of the shock. Two theories, the cellular and the humoral, are thus to be considered, but I am of opinion that they are not as irreconcilable as many investigators tend to believe. Both of these are certainly supported by indisputable evidence and consequently each seems perfectly justified in certain cases. Hence it is evidently necessary to bring them into harmony. However keen may be the interest in knowing where the primary reaction takes place, unquestionably the main thing is to ascertain of what this reaction consists; and it may be presumed that its intimate nature will be, for technical reasons, more easily and successfully investigated in a humoral medium than in a cellular protoplasm. I shall, therefore, try to show that all of the objections raised against the humoral theory are not equally convincing, and that the experiments planned in the direction of humoral analysis are particularly capable of suggesting correct and useful ideas.

In order to explain as clearly as possible the problem of anaphylaxis, it would probably be well to follow chronologically the successive discoveries as they have been linked one with another. But I shall not presume to review, in detail, ideas which you know as well as I do, and perhaps better. Indeed, among the countries which have supplied a large amount of information regarding anaphylaxis, America stands high. American writers, by their number as well as by the value of individual investigations, have taken a prominent part in, and greatly contributed to, the success of the study. Every one knows what we owe to the researches of Theobald Smith, Gay, Southard, Rosenau, Anderson, Frost, Lewis, Auer, Pearce, Eisenbery, Weil, Wells, Osborne, Coca, Schultz, Manwaring, Zinsser, and many others.

As you remember, the term " anaphylaxis " originated with Richet, and was intended to designate the strange fact that an organism subjected to a first injection may become highly susceptible to, instead of immune from, the action of certain poisons, such as the liquid extracted from the body of the mussel or from the tentacles of actinia. Far from protecting the animals, a first injection of such toxic matters rendered them, for a long period, hypersensitive to a subsequent injection. Although striking, this discovery acquired its whole value and significance only through the researches of Arthus, Rosenau and Anderson, who proved that non-toxic albuminoids could give rise to closely allied phenomena. As Arthus showed, horse serum causes no local nor general disturbance when injected subcutaneously into normal rabbits; but when injected into rabbits who have been previously submitted to injections of the same serum, the results are totally different; at the point of inoculation, œdema, induration and occasionally gangrene are observed. Furthermore, a rabbit previously and repeatedly injected may die very rapidly, showing convulsions, and extreme dyspnœa, when intravenously injected with a dose of horse serum harmless to normal animals. Rosenau and Anderson, and soon after, Otto, made a careful study of these phenomena, especially as they affect the guinea-pig. Theobald Smith had already shown that guinea-pigs, after having served as tests for anti-diphtheria serum, that is, having received a very small quantity of horse serum, showed themselves, nearly two weeks later, strangely susceptible to the injection of the same fluid. Rosenau and Anderson ascertained that the condition of hypersensitiveness appeared twelve or fifteen days after the first injection; and further, that minimal amounts, even traces of horse serum were sufficient to sensitize the animals. The second injection, or test injection, dangerous when made into the peritoneal cavity, was still more so when given intravenously; even one-tenth of a cubic centimeter of serum might kill the animal. These writers observed also that, after a female guinea-pig has been sensitized, its offspring is found to be in the same condition. The determination of the nature of the substances capable of inducing sensitiveness was soon completed. Foreign sera, milk, sperma, egg-white, microbes, albuminoids extracted from plants, in short, many proteins capable of functioning as antigenic substances, that is, of giving rise to antibodies, sensitize in very small doses, but lose this property if subjected to coagulating or disintegrating influences before being injected. Carbohydrates, fats, lipoids, do not sensitize; as is well known, they do not belong to the category of antigens.

It was thus readily conjectured that the appearance of sensitiveness must have a connection with the production of those antibodies which characterize immunity. This idea coincided with the important findings, first, that sensitizing substances are antigens; secondly, that sensitiveness does not appear until several days after the inducing injection. Moreover, the capacity of sensitizing, and that of killing at the time of the second injection, belong to one and the same substance. Thus, it may be assumed that the first injection produces antibodies, and that the conflict between these antibodies and the same reinjected antigen produces the disorders. It may thus be understood why anaphylaxis, like antibodies, is highly specific. As is well known, this view of the important rôle of antibodies in anaphylaxis received unquestionable confirmation when Nicolle, Gay and Southard, and Otto, discovered *passive anaphylaxis,* the serum of animals in a condition of sensitiveness injected into normal animals transmitting to them the same condition. Just as there exist two different aspects of immunity, similarly an active and a passive sensitiveness must be distinguished. A close parallelism exists between the sensitizing capacity of an immune serum and its

content of antibodies. A very small dose of the serum of highly immunized animals can confer the sensitiveness. Just as is observed in immunity, passive sensitiveness appears more rapidly than active sensitiveness. The nature of the antibody responsible for the production of sensitiveness varies, as may be expected, with the nature of the antigen under experiment. In the case of red corpuscles, the antibody concerned is the hæmolytic one; in the case of a foreign serum, milk or egg-white, it is in all probability the precipitin which, as a matter of fact, confers also the avidity toward alexin or complement. Treated *in vitro* with the corresponding antigen, an immune serum loses proportionally its power of inducing sensitiveness towards the same antigen. A quite analogous phenomenon may occur *in vivo;* in fact, the sensitiveness of an animal possessing a specific antibody may disappear through the neutralization of the latter when the corresponding antigen is injected, and this is an essential reason, if not the only one, for the condition known as anti-anaphylaxis. As is well known, a guinea-pig sensitized to a given antigen, and which, on being injected with an infralethal dose of the latter, suffers from the shock but recovers, will subsequently resist quantities of the same antigen much larger than the fatal dose. In such a case, anti-anaphylaxis appears as a veritable desensitization due to the neutralization of the antibody requisite for the shock. But, as we shall see further, still other factors may have as an effect the conferring of a condition of anti-anaphylaxis; the susceptibility to the shock itself may decrease.

To sum up, in all of the investigated cases of anaphylaxis depending on the intervention of an antigen, the interaction antigen-antibody represents the primary cause of the accidents. This notion, of course, does not include the hypothesis—which we shall find to be not groundless—that some substances may be capable of developing the shock in spite of their not belonging to the category of antigens or antibodies.

What are the symptoms of anaphylaxis? On this point, we are indebted to American scientists for the greater part of the information collected. We owe to Rosenau and Anderson a careful study of the shock. This was soon followed by the contributions of Gay and Southard dealing especially with the post-mortem condition and by the very accurate study of Auer and Lewis. As you know, when a sensitized guinea-pig receives the test injection, within one or two minutes it begins to be excited, scratches itself, jumps convulsively, coughs, then staggers and finally falls over, showing extreme dyspnœa; the symptoms are those of asphyxia. At the autopsy, a dominant pathological finding is the inflated condition of the lungs, due, as Auer and Lewis have shown, to a contraction, peripheral in origin, of the smooth musculature of the bronchioles, the effect of which is that air can be admitted into the chest by violent inspiratory efforts, but can not be expelled. The liver is much congested and swollen. This symptom is particularly evident in dogs, as Manwaring has pointed out. There is also a surprising persistence of the heart beat, especially of the auricles. In many cases, there are small hemorrhages, scattered in the lungs and other tissues, due in all probability, according to Gay and Southard, to lesions of the endothelium. Other important symptoms of the shock consist in a fall of blood pressure and temperature, a lowering of blood coagulability and of the number of circulating leucocytes and platelets. That the smooth musculature is affected in the shock is proved beyond any doubt by the remarkable researches of Schultz and of Dale, which showed that fragments of small intestine or uterus from sensitized guinea-pigs, immersed in Ringer's solution, contract when there is added to this bath some of the antigen towards which the animal has been sensitized. This experiment undoubtedly explains the fatal bronchiole muscle contraction causing asphyxia. Dale has found recently that if the lungs of an anaphylactic guinea-pig are removed from the body and perfused clear of blood with Ringer's saline solution, addition of a trace of the specific antigen to the perfused fluid causes an immediate contraction of the bronchioles, so intense that air cannot be forced through them. The effect, like the sensitiveness itself, is perfectly specific.

The part played by the smooth musculature in the shock is evident, but, as has been already said, other cells also appear to be affected, especially the endothelial cells, and perhaps also the heart tissue. Since several tissues are involved in the shock, it is easily conceivable that the immediate cause of death may vary with the nature of the tissue most injured, and consequently that the symptoms elicited in different animal species may be not altogether identical. In general the anaphylactic process is the same, but different aspects may be assumed. Smooth musculature is gravely affected both in the guinea-pig and the rabbit, but in the guinea-pig the muscles most markedly implicated are those of the bronchioles, whereas in the rabbit one observes particularly, according to Coca, constriction in the muscular coat of the pulmonary arterioles, the effect of which is to bring about an obstruction to the pulmonary circulation. In dogs, as Manwaring has pointed out, the liver plays a very important part in the shock; the greater portion of the blood is suddenly accumulated in this organ as an effect of an influence exerted on the endothelial cells of its capillaries.

However, these are simply differences in the order of susceptibility of the different tissues concerned in the shock, and thus the discrepancies in detail revealed by the different animal species need not deter us from the opinion that, in the main, the anaphylactic process is everywhere the same as regards its fundamental character. As a matter of fact, the symptoms of shock, though always analogous, are not absolutely identical even in one and the same species, whatever antigen be used. This point has, perhaps, received too little attention. For instance, guinea-pigs sensitized towards horse serum or foreign corpuscles do not, when succumbing to the effects of the injection of the corresponding antigen, show exactly the same lesions. Thus, the tendency to pulmonary hæmorrhages is ordinarily much more marked in the guinea-pig which receives foreign blood corpuscles; sometimes, just before death, a bloody serum or even drops of blood flow from the nostrils. Probably the anaphylactic poison, although resulting in all cases from a similar reaction, is not always

exactly the same, the adsorption properties of the different antigens being not absolutely identical. It being thus established that certain cells are particularly involved and that the shock is due to an antigen-antibody interaction, the question immediately arises as to the primary site of this interaction. Does the latter occur in the sensitive cells themselves, or does it take place in the circulating humors, producing in them some toxic substance capable of reaching subsequently the cells and injuring them? We now approach one of the most disputed points. The cellular theory has found its supporters especially in America and in England, chief among them being Richard Weil, Coca, and Dale. You will recall their main arguments. A very striking one consists in the fact that, at least in the guinea-pig and in the case of many antigens, passive hypersensitiveness is not communicated at once by the injection of the sensitizing serum, as ought to be expected from analogy with passive immunity. For example, susceptibility to horse serum in the guinea-pig appears only some hours after the intravenous injection of the immune serum active towards this antigen, and much later when the same serum is injected subcutaneously. In order to explain this singular fact, first observed by Rosenau and Anderson, Gay and Southard, Doerr and Russ advanced the hypothesis that the antigen-antibody reaction has dangerous consequences only when it takes place in the tissue cells themselves. As a matter of fact, this condition seems to be easily fulfilled because, as was shown by Fenyvessy and Freund, Manwaring, Coca, Weil and others, a large amount of the antibody injected into the circulation rapidly disappears from the blood, being in all probability anchored by the cells. Weil thinks that sensitiveness is caused exclusively through the agency of the absorbed antibody, and that circulating antibodies, on the contrary, exert a protective influence by uniting with the antigen before the latter can reach the cells. To sum up, the interaction, dangerous when it occurs in the cells, is innocuous when it takes place in the blood. The researches of Schultz and Dale, showing that smooth musculature even when freed from its blood is susceptible to the antigen, distinctly support this notion. Nevertheless, I am of opinion that, however correct this view may be in many cases, it cannot be generalized, and that it would be entirely unjustifiable to deduce from it that all of the experimental results dealing with the production *in vitro* of anaphylatoxins ought to be wholly disregarded, as being in contradiction to the cellular theory of anaphylaxis. Such an undue generalization of this assumption that the shock depends upon the occurrence of the antigen-antibody interaction in the cells of vital organs, no disturbance being caused by interaction with a circulating antibody, is to be found, for example, in a paper recently published by Dale, who notes that the subcutaneous injection of the antigen with or soon after the antibody, is uniformly without effect, at any rate in guinea-pigs, and that, as a rule, attempts to produce *in vitro* a real anaphylatoxin through the interaction of antigen and antibody, have failed.

Aside from the fact that in the dog and in the rabbit the sensitizing serum confers sensitiveness without any delay, it may be argued, as regards the guinea-pig, that Dale takes no account of the fundamental experiment showing that passive anaphylaxis towards red corpuscles establishes itself at once. A guinea-pig intravenously injected with rabbit's red corpuscles succumbs to the shock if the corresponding guinea-pig immune serum has been injected into the vein immediately before, or even if it is administered soon afterwards. A similar result is observed when a guinea-pig receives corpuscles which have been treated *in vitro* by the heated corresponding immune serum, and have thus absorbed the specific antibody. Evidently, under such circumstances, the blood plasma instead of the cell protoplasm, is involved in the conflict with the complex antigen-antibody already developed. Furthermore, such rabbit's corpuscles treated with the specific antibody produce *in vitro* a very active anaphylatoxin, when added to fresh normal guinea-pig serum. Hæmolysis having occurred, the mixture is freed from the corpuscle's stromata by centrifuging and pipetted off; this clear fluid produces a typical shock. It cannot be doubted that the symptoms due to the injection of the corpuscle-antibody complex are of the true anaphylactic nature; the circumstances under which they are elicited are wholly in harmony with the definition of anaphylaxis. Now, since the anaphylatoxin produced *in vitro* by mixing such corpuscles with fresh serum brings about exactly the same shock, its connection with typical anaphylaxis is obvious and its significance cannot be questioned.

I know the objection has been raised that the toxic fluid alluded to contained free hæmoglobin and that substances liberated by injured cells may give rise to disturbances having nothing to do with anaphylaxis. But the objection is untenable, since no poison is generated, in the absence of the antibody, when normal guinea-pig serum is added to corpuscles which have been hæmolyzed by distilled water or freezing. It is quite certain that the cellular theory of anaphylaxis cannot be applied to the case of red corpuscles serving as antigen.

Consequently, as regards the primary site of the anaphylactic reaction, neither the humoral nor the cellular theory can be exclusively accepted. Each of them may be true, and it depends upon the antigen. Sometimes the perturbing substance which represents the immediate cause of the shock is engendered in the blood, sometimes it can appear only in the cellular protoplasm, the previous fixation of the antibody by the cells being in such cases a necessary condition.

Is it conceivable that the nature of the various antigen-antibody complexes is so different that they are capable of producing the poison sometimes in the blood, sometimes only in the cells? The discrepancy can be explained if one supposes that the complexes are involved in an adsorption phenomenon occurring between them and some constituents of the medium, the composition of the latter being thus disturbed. It is a recognized fact that adsorption phenomena are easily influenced, that they depend, for instance, on the alkaline or acid reaction, the action of salts, the nature of the colloidal substances present; and it has likewise been proved that different substances capable of adsorption are not necessarily influenced in the same manner or to the same extent by a given

factor. Next, it may be presumed that, as complexes containing different antigens do not possess, in all probability, quite identical absorbing properties, such properties will require, to be clearly manifested, special conditions not likely to be the same for all of the complexes; in other words, a given complex will adsorb best in a given medium, some complexes operating better in a cellular, others in a humoral one. To sum up, the question as to the primary site of the interaction does not appear to have such fundamental importance as has been asserted. Whatever may be the site, the chief problem is to elucidate the nature of the interaction. In all probability, the underlying mechanism of the shock consists in the production of a toxic principle, derived, under the influence of the antigen-antibody complex, from the constituents of the surrounding medium, humoral or cellular, a principle to the presence of which the typical disturbances of the sensitive cells are due.

Were the significance of the anaphylatoxins prepared *in vitro* accepted by the adherents of the cellular hypothesis, the views just expressed could serve as a connecting link between the two otherwise conflicting theories. But such is not the case. According to many writers, the anaphylatoxins, being produced *in vitro* through the agency, not of cells, but of serum, cannot represent the real factor of anaphylaxis. No theoretical reasoning, however, can prevail against an experimental fact, and, as I reminded you before, the mixture of fresh guinea-pig serum and red corpuscles loaded with the specific antibody represents a true anaphylactic poison. Moreover, with this unquestionable anaphylatoxin is connected the one obtained by Friedberger in treating specific precipitates with fresh serum. Considering not alone the similarity of the symptoms elicited, but also the striking analogy between the methods of preparation, taking into account, for example, that in both cases the serum used to produce the poison must be fresh and is rendered inactive when heated to 55° C., one cannot refrain from concluding that the important facts discovered by the supporters of the cellular theory must be brought into harmony with the belief in the unquestionable significance of the several anaphylatoxins which have been described. Whatever may be the site, intracellular or intravascular, of its production, the poison called anaphylatoxin is the causal agent of the shock, the etiological problem resolving itself into that of the genesis of the anaphylatoxin, or, of the anaphylatoxins, the plural allowing the hypothesis that the poison may vary to a certain extent when the antigens are different.

What is the origin of the anaphylactic poison? As is well known, its appearance has been ascribed to the action of proteolytic ferments which might liberate toxic products at the expense of albuminous matters. This conception has been supported chiefly by Vaughan and Wheeler. Wells, Biedl and Kraus pointed out the real analogy existing between the symptoms of anaphylaxis and the effects of the injection of peptone. Assuming likewise that a digestion of the antigen furnished the poison, Friedberger thought that the proteolytic influence was exerted by the alexin or complement with the coöperation of the previously absorbed specific antibody. All of these conceptions, based on the hypothesis that the poison is materially derived from the antigen, are liable to serious objections. The presence of proteolytic ferments in the blood after the first injection of an antigen is not concomitant with the condition of sensitiveness. There exists not the least proof of an enzymatic property on the part of alexin or complement. The phenomena due to the lytic influence of alexin, as bacteriolysis and hæmolysis, are not accompanied by modifications indicating any chemical decomposition of albuminoids.

The foregoing theories being inadequate, it might be surmised that the toxic substance, instead of being derived from the antigen, is evolved from the serum, or from the cellular protoplasm, with which the antigen-antibody complexes are brought into contact, these media being modified in their constitution as a consequence of the adsorbing properties of the complexes. This idea originated with researches which, in conjunction with Gengou, I reported nine years ago. We described a peculiar phenomenon called by us the coagglutination of red corpuscles. We were able to show that the complexes antigen-antibody may possess very strong adsorption properties which are capable of being displayed even toward cells. We, therefore, suggested the hypothesis that perhaps the appearance of toxicity in a serum treated by such a complex might result from an adsorption phenomenon. The composition of blood plasma is very complex and still but little known. The number of active substances contained in it, taking part in the regular working of physiological functions, is undoubtedly considerable. All of them are necessary to the physiological equilibrium, and it may thus be presumed that the removal of certain of these principles may communicate abnormal properties to the plasma and even render it dangerous. This view was soon upheld by several writers, especially Doerr and Ritz, and some attempts were made to produce a toxin by adding to fresh serum certain adsorbing substances, such as kaolin. But the results were inconstant, and not convincing. The correctness of the idea was finally proven in 1913, when I discovered that ordinary agar is a very suitable and efficient adsorbent. I saw that fresh guinea-pig serum, when incubated even with traces of an emulsion of agar, is converted into a powerful anaphylatoxin. For example, twenty-five or fifty centigrams of agar, dissolved by heating in one hundred cubic centimeters of physiological salt solution, give when cool a flaccid mass which is easily transformed by shaking into a viscous fluid, to which five parts of fresh serum are added. Incubated for about two hours, the mixture is centrifugalized; the agar is wholly deposited, the clear supernatant fluid pipetted off and injected in a dose of from 3 to 5 c. c. into the jugular vein of a guinea-pig, kills it in a few minutes, the symptoms exhibited being precisely identical with those of anaphylactic shock. Excitement, jumping, cough, convulsions, symptoms of asphyxia, inflation of the lungs, ecchymosis, long duration of heart pulsation, very slow or no coagulation of the blood—nothing is missing. Of course, normal serum, not treated with agar and injected in the same dose, produces no appreciable effect. The agar-anaphylatoxin resembles closely the anaphylatoxin obtained by Friedeman or

Friedberger by contact of serum with antigens in the presence of the antibodies. No toxin is obtained when agar is mixed, not with fresh serum, but with serum previously heated to 55° C. Friedberger has likewise pointed out that heated serum is not suitable for obtaining his anaphylatoxin. But if anaphylatoxin be prepared by means of unheated serum and agar, it does not lose its toxicity when heated to 55-60° C., and the same fact may be observed with regard to the anaphylatoxin obtained, for example, by contact of fresh serum and foreign red corpuscles loaded with the specific antibody. The fact that anaphylatoxin resists such temperature excludes the possible objection that the coagulating principle of the serum, fibrin-ferment or thrombin, may be responsible for the toxic effects, since it is destroyed much more easily. The activity of thrombin decreases so rapidly, that normal serum is toxic for only a very short period after its production. On the contrary, when kept at the room temperature, the agar anaphylatoxin is still toxic after several months. Calcium salts are not requisite for the production of the poison, the latter appearing as well in the presence of sodium oxalate. Saturation with carbonic oxide gas favors the production of the poison.

My observations on agar-anaphylatoxin have been confirmed by Nathan, Loewit and Baeyer, Ropaczewski and Muttermilch, and in this country by the remarkable researches of Novy and de Kruif. Nathan even found that agar could be replaced by starch, although the effect is not so striking. Ropaczewski asserted that sodium pectate had the same property. Zunz and Gelat observed that horse serum could also be rendered toxic by agar. Novy and de Kruif found that rat serum was still more suitable than guinea-pig serum.

Soon after I had made known the agar-anaphylatoxin, Friedberger tried to reconcile this discovery with his own theory by assuming that agar contained some albuminous matter which was digested under the influence of alexin and thus liberated toxic products. But at the International Medical Congress held in London in 1913, I was able to refute that objection by demonstrating that starch entirely loses its property of inducing the formation of anaphylatoxin when liquified by the saccharifying diastase, which of course does not act on albuminous matters, and, consequently, the toxigenic property of starch is not due to such substances. Furthermore, I was able, in coöperation with Dr. Zunz, to obtain a purified agar containing no trace of nitrogen but which still induced the formation of an anaphylatoxin. Similarly, Ropaczewski asserts that the pectate which gave positive results was quite free from nitrogen.

Not every colloid or suspension of the particles is capable of converting the serum into anaphylatoxin. As Nathan showed, a suspension, not a solution of inulin, is apt to act as agar does, but I did not succeed when I tried gelatinous silicic acid, animal charcoal, gum arabic or some gelatinous precipitates such as tricalcic phosphate; moreover, the results obtained by some writers with kaolin have been disputed.

Such being the facts, the conclusion I felt entitled to state, and against which I think nothing could be objected, was, that the capacity of agar to convert, by a simple phenomenon of adsorption, the fresh serum into anaphylatoxin, demonstrates that the poison does not proceed from any digested antigen, but is derived from the normal constituents of the organism itself and that the frequent appearance of anaphylatoxin in the course of immunity phenomena arises simply from the fact that the complex antigen-antibody is endowed with adsorption properties quite analogous to those so distinctly displayed by agar.

Is anaphylaxis, then, the reverse of immunity? Evidently not. It is a secondary phenomenon, certainly harmful, but which, far from betraying an excessive and unwonted receptivity, testifies on the contrary that the factors of immunity are operating. In short, it is an accident in the course of the defence.

Unhappily, although those results are already seven years old (if the period of war, so unfavorable to scientific work, is to be taken into account), the intimate nature of the reaction occurring between agar and serum is still very mysterious. Agar has been supposed to remove an antitryptic agent and thus allow a desintegration of the albuminous constituents of the serum, but the attempts of Zunz and myself, and of Novy and de Kruif to test this hypothesis by detecting an increase of amino-acids in the agar anaphylatoxin did not favor this assumption. Novy and de Kruif think that the evanescent toxicity of blood just before or soon after spontaneous coagulation is to be attributed to a principle similar to the anaphylatoxin. I do not believe this opinion to be correct. The toxicity of clotting blood or of quite fresh serum is sufficiently explained by the presence of thrombin, the coagulating activity of which decreases, just as does its toxicity, very rapidly. True anaphylatoxins are stable. The same toxicity of recently clotted blood may appear in any serum, at any moment, provided it contains a sufficient amount of serozym, simply by adding, in the presence of calcium, platelets or cytozym to it, fresh thrombin being thus produced. But cytozym and calcium salts are not, so far as I could ascertain, necessary to the production of anaphylatoxin. In short, the conversion of serum into a toxin by the mere influence of agar has not been explained.

I think that sufficient evidence has been afforded by the foregoing considerations in favor of the view that the conception of anaphylatoxin, even when the poison is obtained through the agency of a non-antigenic substance such as agar, contributes very much to the understanding of the shock occurring in animals actively or passively sensitized towards an antigen. The connection between the different facts appears to be satisfactorily established. But it seemed desirable to make it still more evident. Perhaps this need has been met, to a certain extent at least, by researches carried on by me during the war concerning antianaphylaxis. I found that agar anaphylatoxin may be produced, not alone *in vitro,* but also *in vivo,* simply by injecting intravenously into a guinea-pig a small amount of agar emulsion. Under such conditions, the interaction giving rise to the poison occurs in the circulation itself. The same fact was observed, independently, of

course (no communication being then possible between invaded Belgium and civilized countries), by the American scientists, Novy and de Kruif, and also by Zunz. But I observed further that when a guinea-pig is given, intravenously, agar in somewhat less than the lethal dose, it becomes capable, after its recovery from the shock, of resisting a quantity of agar at least sufficient to kill an unprepared guinea-pig. This is undoubtedly an example of antianaphylaxis, but one which of course could not be interpreted by the neutralization of an antibody, since no antibody is in question in the case of agar. Now, it must be noticed that, even in the case of anaphylaxis due to the intervention of antigen and antibodies, the condition of enhanced resistance or antianaphylaxis is not entirely explained by the assumption, mentioned above, that the antibody has been neutralized by the injected antigen. The latter influence certainly plays an important rôle, but it must be pointed out that, aside from this protecting factor, the animal becomes more able to withstand the shock itself. This may be proved by experimenting, for example, with red corpuscles which have absorbed the specific antibody. An infralethal dose injected intravenously develops a curable shock. Then, after recovery, the animal is more able than before to withstand the lethal dose. In such a case the acquired tolerance clearly results from a physiological response to the poisonous action of anaphylatoxin produced *in vivo*. It could not be alleged that the animal resists better the second injection because the requisite antibody has been previously neutralized; in fact, antibody is never lacking, since it is introduced, together with the red corpuscles, by the second injection as well as by the first.

Now, how is this new aspect assumed by antianaphylaxis to be interpreted? I observed the following fact: Large guinea-pigs receive in the jugular an injection of half a cubic centimeter of agar-suspension at 0.25 per cent, a dose of a little less than the lethal one. They recover and are thereby enabled to resist, on the following day, the injection of one cubic centimeter of the same suspension, that is, of at least the lethal dose. Several hours later they are bled, and their serum is subsequently tested for its ability to be converted into anaphylatoxin, by the addition of agar in the ordinary amount of five parts of serum for one part of agar. Mixtures similar to these but containing serum of normal unprepared guinea-pigs are then employed. The result is, that the serum derived from the guinea-pigs treated with intravascular injections of agar has lost almost completely the capacity of producing *in vitro* anaphylatoxin under the influence of agar, whereas normal serum, as we know, is regularly endowed with this property. It is a quite remarkable fact that the same serum of prepared guinea-pigs gives, when treated with rabbit's red corpuscles loaded with the specific antibody, the same negative result; the liquid does not become toxic. Thus a very close connection is established between the different anaphylatoxins, prepared either with or without the interference of an antigen-antibody complex, and thus the experiments concerning agar gain a wider significance for the understanding of anaphylaxis as observed under ordinary circumstances. I may add that, similarly, the serum of guinea-pigs which have recovered from an injection of foreign red corpuscles loaded with antibody is markedly less capable than normal serum of giving anaphylatoxin when brought into contact with the same complex antibody-corpuscles. We consequently reach the conclusion that antianaphylaxis is due, at least partly, to a modification of the serum, consisting probably in the presence of some substance capable of protecting from the immediate factor of the shock, that is, from the anaphylatoxin.

It would be very interesting to investigate whether any interaction can be detected between the anaphylatoxin and the tissues, such as smooth musculature, which are particularly susceptible to the shock. We are working in Brussels on these lines, but such experiments are difficult owing to the fact that the tissue extracts are, as we know, poisonous in themselves. Perhaps some results will nevertheless be attained, but we must not anticipate. My only desire to-day has been to point out that it would be quite unjustifiable, however correct may be many assumptions of the cellular theory, to disregard the humoral side of the anaphylactic phenomena, and I feel particularly glad that several American writers, among them Novy and de Kruif, have successfully contributed to the discoveries in that direction. Anaphylatoxin has undoubtedly much to do with real anaphylaxis and even did such a connection not exist in reality, the capacity of normal serum to be converted readily into a poison by the action of agar is an unexpected and very striking fact, enigmatic enough to deserve, at any rate, the serious attention of the physiologist.

NOTES ON NEW BOOKS

The Oxford Medicine. By Various Authors. Edited by Henry A. Christian, M. D., and Sir James Mackenzie, M. D. Vols. I and II. (*London and New York: Oxford University Press, 1920.*)

It is with unusual interest that one approaches the volumes of the new Oxford Medicine. Appearing as they do at the height of an era of new ideas in medical thought, and sponsored by prominent and popular editors, one can be sure that they will contain much of interest and value. However, before attempting any review of this work one is tempted to collect his thoughts as to the ideal system or compendium of internal medicine. What should be its form, content, and purpose? One likes to think of it as a grand collection of information about disease, a storehouse of knowledge into which one may delve with the reasonable certainty of finding information which will clear up a doubtful point. It, therefore, is interesting to find that the Oxford system is planned along other lines—it is reflective rather than statistical, autobiographical rather than objective, and in general it tends more to bring out the point of view of an individual in regard to certain phases of medicine than to present an impartial résumé of what has been established as fact. Under these circumstances one discovers naturally an uneven quality in the various articles. Most of them are good, some are superlative, while a few are distinctly inferior.

Volume I contains a series of interesting articles on the general subjects of diagnosis and the relations of the fundamental sciences—biology and chemistry—to clinical medicine. The reflections of Christian and Mackenzie on the present and future of medicine form a pleasant introduction to the succeeding sections. The summary of protein chemistry in relation to disease by Van Slyke, acidosis by Henderson, and respiration by Peabody are perhaps the outstanding articles in this volume. The sections dealing with infection are less pleasing. The one on focal infection is especially replete with statements which surely cannot be acceptable to many physicians. Our own reaction is decidedly against the elevation of the doctrine of so-called focal infection to the dignity of a cult. It seems unnnecessary to confuse the well established principles of sepsis by assumptions which, at best, are dubious.

Volume II, dealing with diseases of the lungs, heart and blood, contains many excellent articles. Those on leukæmia and on the diseases of the lungs stand out on account of their clearness and precision. One is surprised at the special article on massive collapse of the lung, a condition quite outside our own experience as a distinct disease entity. One may also criticize the article on asthma, in so far as it presents the subject from a narrow point of view only—that of anaphylaxis.

On the whole, the impression gained from the first two volumes of the Oxford Medicine is pleasant. As a summary of prevailing views in medicine it is of value, though one can in no sense think of it as a monumental work.

A. L. B.

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DETERMINATION OF THE BASAL METABOLISM FROM THE CARBON-DIOXIDE ELIMINATION

By JOHN T. KING, JR.

WITH A STATISTICAL NOTE

By RAYMOND PEARL

(From the Medical Clinic of The Johns Hopkins Hospital)

Estimation of the basal metabolism is evolving rapidly into a common laboratory and even bedside procedure. Since it was found by Lusk, Benedict, DuBois and others, that measurement of the oxygen and carbon-dioxide gas exchange offers a method for calculating the heat production that compares favorably with direct observation in the chamber calorimeter, many gas exchange methods have been developed. At least three of these are based upon the measurement of both oxygen intake and carbon-dioxide elimination. The "Tissot" method described fully by Boothby and Sandiford,[1] is an "open" method, the patient inhaling fresh air through a valve and the exhaled air being subjected to gas analysis for remaining oxygen and eliminated carbon-dioxide. The "universal respiration apparatus" has been evolved under Benedict[2] into a "portable" apparatus, a "closed" system which affords a method for direct measurement of oxygen consumption by volume and also for the measurement of carbon-dioxide elimination by weight. A combined chamber and respiration apparatus is in use at New Haven.[3] It has been customary, when both oxygen and carbon-dioxide figures are obtained, to calculate the heat production in calories from the oxygen consumption, taking into consideration the respiratory quotient.

More recent methods deal with the oxygen consumption only, a respiratory quotient of 0.82 being assumed. Apparatuses for the measurement of oxygen alone have been proposed by Benedict and Collins[4] and by Jones,[5] and simplified forms of apparatus for bedside use are being manufactured by commercial houses on their own initiative.

Although criticism of any method may, to a certain extent, be justifiable, some helpful information can undoubtedly be obtained through the use of any one of them by a careful observer. The attempt is being widely made to find the most accurate, stable and simple method which at the same time is

[1] Boothby and Sandiford: Laboratory Manual of the Technic of Basal Metabolic Rate Determination. Saunders, Philadelphia, 1920.

[2] Benedict, F. G.: A Portable Respiration Apparatus for Clinical Use. Boston Med. and Surg. Jour., 1918, CCXXVIII, 667.

[3] Barbour, H. G.: Antipyretics. I. The Benedict Respiration Chamber at the New Haven Hospital. Arch. Int. Med., 1919, XXIV, 611.

[4] Benedict and Collins: A Clinical Apparatus for Measuring Basal Metabolism. Boston Med. & Surg. Jour., 1920, CLXXXIII, 449.

[5] Jones, H. M. A Simple Device for Measuring Basal Metabolism. Jour. Am. Med. Assn., 1920, LXXV, 538.

hygienic and relatively inexpensive for use in routine clinical examinations.

So far, it has not been suggested to make use of a simple measurement of the carbon-dioxide elimination as an index to the metabolic rate. Barbour,[5] however, who uses a combined box and Benedict respiration apparatus, states that he has found the carbon-dioxide elimination to be less variable than the oxygen consumption and has therefore been accustomed to calculate heat production from carbon-dioxide elimination. It seemed to us to be worth while to find out how valuable would be an index to the metabolic rate obtained by measuring carbon-dioxide elimination alone.

In order to determine this point, it seemed the best procedure to study the original chamber calorimetric work. By so doing, we are able to compare oxygen consumption with carbon-dioxide elimination and also to compare each of these factors with the heat production, as measured directly.

For this purpose 27 experiments have been analysed, 10 being taken from the publication of Benedict and Carpenter[6] and 17 from work by Sonderstrom, Meyer and DuBois.[7]

[6] Table I.
[7] Table II.

Each experiment represents an hour's observation, though in some repeated tests were made upon the same subject. Only observations upon resting subjects in the post-absorptive state were selected. In the case of the Benedict and Carpenter observations, the more recent DuBois height-weight formula for computing body surfaces was applied to data furnished in the published protocols.

The ten protocols of Benedict and Carpenter were analysed by the writer in Table I as follows: The average carbon-dioxide elimination per square meter per hour was determined (Col. A); the average oxygen consumption was determined (Col. B); and the average heat production was determined (Col.C). On the basis of these averages, the deviation of carbon-dioxide from its average was calculated for each experiment. The deviations of oxygen consumption and of heat production from their respective averages were also calculated for each experiment. The carbon-dioxide deviation from its average was then compared directly with the deviation of heat production from its average in each experiment. It was found that the average difference between carbon-dioxide variations and calorie variations was 4.93 per

TABLE I

DATA FROM BENEDICT AND CARPENTER

METABOLISM AND ENERGY TRANSFORMATION OF HEALTHY MEN

Pub. Carnegie Inst., 1910

Name	Height in cm.	Weight in kgm.	Body surface (height-wt. form)	Direct calorimetry: A		B		C		Deviation of CO_2 from average (in gms.)	Deviation of O_2 from average (in gms.)	Deviation of cals. from average (in cals.)
				CO_2 per hr. (gms.)	CO_2 per sq. m. per hr. (gms.)	O_2 per hr. in gms.	O_2 per sq. m. per hr. (gms.)	Cals. per hr.	Cals. per sq. m. per hr.			
C. R. Y.	170	67.8	1.78	29.35	16.45	25.9	14.55	97.2	54.6	+2.39	+2.36	+9.8
A. H. M.	179	65.3	1.83	24.80	13.56	20.5	11.20	81.3	44.5	—0.50	—0.99	—0.3
A. L. L.	166	67.5	1.75	23.50	13.42	17.8	10.17	81.0	46.3	—0.64	—2.02	+1.5
H. R. D.	171	58.2	1.69	25.65	15.20	25.6	15.15	74.65	44.2	+1.14	+2.96	—0.6
H. R. D.	171	58.2	1.69	22.55	13.32	17.3	10.23	67.9	40.2	—0.74	—1.96	—4.6
H. C. K.	181	73.0	1.93	25.95	13.45	23.6	12.23	87.65	45.4	—0.61	+0.04	+0.6
H. C. K.	181	73.0	1.93	25.45	13.20	22.1	11.45	82.25	42.6	—0.86	—0.74	—2.2
H. C. K.	181	73.0	1.93	28.70	14.87	25.0	12.95	83.35	43.2	+0.81	+0.76	—1.6
H. C. K.	181	73.0	1.93	26.40	13.68	22.3	11.55	85.85	44.5	—0.38	—0.64	—0.3
H. C. K.	181	73.0	1.93	26.10	13.62	24.1	12.48	83.4	43.2	—0.54	+0.29	—1.6
Total					140.67		121.96		448.7			
Average					14.06		12.19		44.8			

Name	Per cent variations of CO_2, O_2, and calories (direct method) computed from above data: Deviation of CO_2 from average		Deviation of O_2 from average		Deviation of cals. from average						Comparison of CO_2 variations with calorie variations and of O_2 variations with calorie variations: Deviation of CO_2 from cals. (per cent)	Deviation of O_2 from cals. (in per cent)
C. R. Y.	+17.0	.....	+19.3	.....	+21.9	.....	.....	.....	.....	.....	—4.90	— 2.6
A. H. M.	— 3.56	.....	— 8.1	.....	— 0.7	.....	.....	.....	.....	.....	—3.49	— 7.4
A. L. L.	— 4.51	.....	—16.5	.....	+ 3.3	.....	.....	.....	.....	.....	—7.81	—19.8
H. R. D.	+ 8.1	.....	+24.3	.....	— 1.4	.....	.....	.....	.....	.....	+9.50	+25.7
H. R. D.	— 5.26	.....	—16.1	.....	—10.3	.....	.....	.....	.....	.....	+5.04	— 5.8
H. C. K.	— 4.37	.....	+ 0.3	.....	+ 1.4	.....	.....	.....	.....	.....	—5.77	— 1.1
H. C. K.	— 6.12	.....	— 6.1	.....	— 4.9	.....	.....	.....	.....	.....	—1.22	— 1.2
H. C. K.	+ 5.76	.....	+ 6.2	.....	— 3.6	.....	.....	.....	.....	.....	+9.36	+ 9.8
H. C. K.	— 2.72	.....	— 5.2	.....	— 0.7	.....	.....	.....	.....	.....	—2.02	— 4.5
H. C. K.	— 3.84	.....	+ 2.4	.....	— 3.6	.....	.....	.....	.....	.....	—0.24	+ 6.0
										Total....	49.35	83.9
										Average .	4.93	8.39

TABLE II

DATA FROM SONDERSTROM, MEYER AND DUBOIS

Arch. Int. Med., 1916, V. 17, part 2, p. 872

		Indirect calorimetry											
		A			B				C				
Name	Body surface (Dubois linear formula)	CO_2 eliminated per hour (gms.)	CO_2 per sq. meter per hour (gms.)	Deviation of CO_2 from average (gms.)	Deviation of CO_2 from average (in per ct.)	O_2 used per hour per sq. m. (Gms.)	Deviation of O_2 from average (Gms.)	Deviation of O_2 from average (in per ct.)	Cals. per sq. m. per hour	Deviation of cals. from average	Deviation of cals. from average (in per ct.)	Deviation of CO_2 from cals. in per ct.	Deviation of O_2 from cals. in per ct.
A. G.	1.67	24.32	14.55	+1.34	+10.01	12.38	+0.45	+ 3.78	41.50	+3.03	+ 8.14	+1.87	— 4.36
A. G.	1.67	25.97	15.50	+2.29	+17.30	12.54	+0.61	+ 5.12	42.55	+4.08	+10.61	+6.69	— 5.49
A. G.	1.67	24.31	14.50	+1.29	+ 9.76	12.71	+0.78	+ 6.50	42.30	+3.83	+ 9.95	—0.19	— 3.45
A. G.	1.67	22.43	13.45	+0.24	+ 1.82	11.90	—0.03	— 0.25	39.80	+1.33	+ 3.46	—1.64	— 3.71
A. G.	1.67	25.06	15.05	+1.84	+13.92	12.20	+0.27	+ 2.25	41.40	+2.93	+ 7.62	+6.30	— 5.37
A. G.	1.67	24.92	14.90	+1.69	+12.80	13.65	+1.72	+14.41	45.30	+6.83	+17.75	—4.95	— 3.34
R. H. S.	1.83	23.21	12.68	—0.53	— 4.01	11.94	+0.01	+ 0.08	36.50	—1.97	— 5.13	+1.12	+ 5.21
R. H. S.	1.83	23.79	12.98	—0.23	— 1.74	12.51	+0.58	+ 4.85	38.10	—0.37	— 0.96	—0.78	+ 5.81
E. F. D. B.	1.91	23.43	12.29	—0.92	— 6.96	12.79	+0.86	+ 7.22	37.00	—1.47	— 3.82	—3.14	+11.04
E. F. D. B.	1.91	23.69	12.40	—0.81	— 6.13	13.18	+1.25	+10.50	38.05	—0.42	— 1.09	—5.04	+11.59
Wm. A.	1.80	25.32	14.08	+0.87	+ 6.59	12.17	+0.24	+ 2.00	38.50	+0.03	+ 0.08	+6.51	+ 1.92
Cardiac Pt.	1.80	24.08	13.38	+0.17	+ 1.29	12.52	+0.59	+ 4.95	38.95	+0.48	+ 1.25	+0.04	+ 3.70
(Restless)	1.80	24.45	13.60	+0.39	+ 2.92	13.19	+1.26	+10.50	40.70	+2.23	+ 5.79	—2.87	+ 4.71
DATA FROM GEPHARDT AND DUBOIS Arch. Int. Med., Vol. XVII, Part 2, p. 902													
Emma W.	1.64	18.22	11.12	—2.09	—15.82	9.85	—2.08	—17.44	33.25	—5.22	—13.56	—2.26	—3.88
(Woman)	....	18.41	11.22	—1.99	—15.07	9.68	—2.25	—18.85	32.87	—5.60	—14.55	—0.52	—4.30
	....	18.55	11.30	—1.91	—14.46	9.61	—2.32	—19.41	33.03	—5.44	—14.14	—0.32	—5.27
	....	18.86	11.50	—1.71	—12.94	10.30	—1.63	—13.68	34.25	—4.22	—10.97	—1.97	—2.71
Total	....		224.50			203.12			654.05			46.21	85.86
Average	....		13.21			11.93			38.47			2.72	5.05

	Direct calorimetry of preceding observations					Comparison of CO_2, O_2 and indirect calorie figures with direct calories		
		C						
Name	Body surface (linear formula)	Cals. per hour	Cals. per sq. m. per hour	Deviation of cals. from average (in cals.)	Deviation of cals. from average (in per cent)	Deviation of CO_2 from direct cals. (in per cent)	Deviation of O_2 from direct cals. (in per cent)	Deviation of indirect cals. from direct cals. (in per cent)
A. G.	1.67	69.44	41.58	+3.52	+ 9.2	+ 0.81	— 5.42	— 1.06
A. G.	....	72.03	43.13	+5.07	+13.3	+ 4.00	— 8.18	— 2.69
A. G.	....	73.58	44.06	+6.00	+15.6	— 5.84	— 9.10	— 5.65
A. G.	....	65.19	39.04	+0.98	+ 2.6	— 0.78	— 2.85	+ 0.86
A. G.	....	70.68	42.32	+4.26	+11.2	+ 2.72	— 8.95	— 3.58
A. G.	....	73.59	44.06	+6.00	+15.6	— 2.80	— 1.19	+ 2.15
R. H. S.	1.83	72.65	39.70	+1.64	+ 4.3	— 8.31	— 4.22	— 9.43
R. H. S.	....	71.40	39.02	+0.96	+ 2.5	— 4.24	+ 2.35	— 3.46
E. F. D. R.	1.91	69.89	36.59	—1.47	— 3.8	— 3.16	+11.02	— 0.02
E. F. D. R.	....	75.52	39.54	+1.48	+ 3.9	—10.03	+ 6.60	— 4.99
Wm. A.	1.80	62.29	34.60	—3.46	— 9.1	—15.69	+11.10	+ 9.18
Wm. A.	....	69.48	38.60	+0.54	+ 1.4	— 0.11	+ 3.19	— 0.15
Wm. A.	....	70.55	39.19	+1.13	+ 2.9	+ 0.02	+ 7.60	+ 2.89
Emma W.	1.64	54.38	33.16	—4.90	—12.9	— 2.92	— 4.54	— 0.66
Emma W.	....	49.73	30.32	—7.74	—20.3	+ 5.23	+ 1.45	+ 5.75
Emma W.	....	46.26	28.21	—9.85	—25.9	+11.44	+ 6.19	+11.76
Emma W.	....	55.75	33.99	—4.07	—10.7	— 2.24	— 2.98	— 0.27
Total	....	....	647.11			80.44	96.93	64.55
Average	....	....	38.06			4.73	5.70	3.79

cent. The average difference between oxygen variations and calorie variations was 8.39 per cent.

An analysis was made in a corresponding manner of the data of Sonderstrom, Meyer and DuBois. In this series the calories calculated by the "indirect method" are also published. It must be remembered that the technique of these early observations with the chamber calorimeter included not only the O_2 consumption and the CO_2 elimination but urinary nitrogen determination as well.

By these means, the nitrogenous metabolism was estimated, and the O_2 and CO_2 figures were partitioned and calculated for protein and non-protein oxidation. The "indirect method" used in connection with the chamber calorimeter may, therefore, be taken as ideal.

The analysis is shown in Table II. It will be seen that the average difference between the indirect calorimetric figures and the direct calorimetric figures amounts to 3.79 per cent. The average difference between CO_2 and direct calorimetric figures is 4.73 per cent. The average differences between O_2 and direct calorimetric figures is 5.70 per cent.

A third series of protocols was studied (Table III), chiefly for the purpose of obtaining a scale of standard figures for the

TABLE III

FROM THE BASAL, GASEOUS METABOLISM OF NORMAL MEN AND WOMEN

BENEDICT, EMMES, ROTH AND SMITH

Jour. Biol. Chem., Vol. XVIII, No. 2, July, 1914

NORMAL MEN

Name	Age	Weight in kgm.	Height in cm.	Body surface (DuBois)	A				B′			C′	
					CO_2 per min. (cc.)	CO_2 per hour (cc.)	CO_2 per hour (gms.)	CO_2 per sq. m. per hour (Gms.)	O_2 per min. (cc.)	O_2 per hr. (cc.)	O_2 per hr. per sq. m. (cc.)	Cals. total (24 hrs.)	Cals. per sq. m. (24 hrs.)
H. W.	19	108.9	198	2.43	326	19,560	38.43	15.82	361	21,660	8,914	2,559	1,053
W. S.	22	88.5	165	1.96	241	14,460	28.00	14.29	289	17,340	8,840	2,017	1,029
O. F. M.	24	85.8	121	1.98	209	12,540	24.62	12.46	265	15,900	8,030	1,827	922
F. G. B.	41	83.1	183	2.05	217	13,020	25.58	12.47	258	15,480	7,551	1,802	879
Prof. C.	36	83.0	169	1.93	199	11,940	23.43	12.17	237	14,220	7,370	1,655	857
J. H. R.	23	82.2	187	2.07	243	14,580	28.62	13.84	282	16,920	8,174	1,978	956
D. H. W.	22	82.1	186	2.06	245	14,700	28.93	14.03	291	17,460	8,475	2,034	987
H. F.	63	82.1	166	1.9	180	10,800	21.42	11.19	236	14,160	7,449	1,615	851
M. H K.	19	79.0	188	2.05	243	14,580	28.62	13.98	276	16,560	8,075	1,944	949
E. G.	20	78.9	184	2.02	262	15,720	30.46	15.09	302	18,120	8,970	2,126	1,053
W. A. M.	23	78.0	183	2.0	213	12,780	25.03	12.52	262	15,720	7,860	1,816	907
Dr. M.	28	75.9	180	1.95	214	12,840	25.22	12.95	273	16,380	8,400	1,877	962
M. Ba.	20	75.0	180	1.94	221	13,260	26.20	13.52	263	15,780	8,130	1,837	947
F. E. M.	38	75.0	164	1.82	209	12,540	24.62	13.53	242	14,520	7,980	1,698	932
J. F. M.	20	74.5	181	1.95	227	13,620	26.78	13.73	269	16,140	8,280	1,878	963
F. A. R.	32	74.4	163	1.8	205	12,300	24.17	13.43	244	14,640	8,135	1,704	948
W. J. T.	22	74.2	183	1.95	206	12,360	24.23	12.43	256	15,360	7,875	1,770	908
F. G. R.	20	74.0	179	1.93	242	14,520	28.64	14.87	267	16,020	8,300	1,914	992
C. D. R.	22	74.0	173	1.88	238	14,280	28.02	14.92	270	16,200	8,620	1,908	1,015
H. R. W.	24	73.9	175	1.89	222	13,320	26.19	13.85	264	15,840	8,380	1,842	974
J. P. C.	23	73.7	169	1.85	186	11,160	21.90	11.85	218	13,080	7,070	1,526	825
A. O. G.	25	73.2	179	1.92	194	11,640	22.86	11.92	271	16,260	8,470	1,835	956
H. W. E.	23	73.0	168	1.83	190	11,400	22.39	12.23	222	13,320	7,275	1,559	852
P. D. F.	23	71.2	176	1.87	219	13,140	25.82	13.83	259	15,540	8,320	1,810	968
C. B. S.	26	71.1	179	1.89	202	12,120	23.83	12.63	244	14,640	7,740	1,700	899
J. H. H.	25	69.1	171	1.81	197	11,820	23.22	12.84	234	14,040	7,752	1,634	903
N. K. W.	35	68.4	166	1.77	196	11,760	23.08	13.06	219	13,140	7,425	1,549	875
B. A. W.	26	67.9	174	1.82	229	13,740	27.00	14.84	280	16,800	9,231	1,945	1,069
K. H. A.	26	66.4	182	1.86	199	11,640	22.87	12.32	238	14,280	7,675	1,654	889
J. R.	27	66.0	182	1.85	201	12,060	23.68	12.82	241	14,460	7,815	1,679	907
M. A. M.	29	66.0	177	1.81	206	12,360	24.26	13.42	242	14,520	8,025	1,695	937
F. P. R.	22	65.1	173	1.78	182	10,920	21.47	12.08	222	13,320	7,480	1,543	867
J. J. C.	27	65.0	175	1.79	190	11,400	22.38	12.49	227	13,620	7,610	1,585	886
E. H. T.	25	64.7	170	1.75	173	10,380	20.38	11.64	217	13,020	7,440	1,499	857
D. M.	22	64.0	171	1.75	187	11,220	22.05	12.62	240	14,400	8,225	1,651	944
M. J. S.	24	63.7	170	1.74	195	11,700	22.98	13.22	237	14,220	8,170	1,647	947
M. Y. B.	20	63.5	172	1.75	207	12,420	24.42	13.97	238	14,280	8,160	1,677	958
R. O. S.	21	63.5	170	1.74	205	12,300	24.17	13.88	228	13,680	7,860	1,619	930
E. P. C.	35	63.2	185	1.85	171	10,260	20.13	10.88	216	12,960	7,001	1,489	805
R. G.	23	62.7	173	1.75	194	11,640	22.87	13.08	227	13,620	7,780	1,590	908
U. R. B.	27	62.6	173	1.75	179	10,740	21.10	12.07	220	13,200	7,540	1,525	872
W. F. M.	21	62.4	180	1.80	221	13,260	26.03	14.48	259	15,540	8,640	1,816	1,009
H. H. A.	22	62.3	164	1.68	179	10,740	21.10	12.57	213	12,780	7,610	1,487	886
H. C. B.	27	62.0	173	1.74	192	11,520	22.63	13.03	239	14,340	8,240	1,653	950
S. A. R.	23	60.8	165	1.67	175	10,500	20.62	12.35	209	12,540	7,509	1,460	873
A. L.	40	60.6	171	1.71	191	11,460	22.52	13.17	225	13,500	7,890	1,576	922
W. G. J.	21	60.5	175	1.74	210	12,600	24.75	14.23	250	15,000	8,621	1,746	1,003
H. L. H.	26	60.5	172	1.72	200	12,000	23.58	13.71	244	14,640	8,511	1,696	985
J. E. F.	21	60.4	172	1.72	202	12,120	23.82	13.87	229	13,740	7,990	1,616	940
J. K. M.	24	60.4	173	1.73	182	10,920	21.44	12.42	223	13,380	7,730	1,549	895
H. B. R.	25	60.2	168	1.68	173	10,380	20.39	12.13	214	12,840	7,640	1,487	885
J. B. T.	20	60.1	171	1.71	209	12,540	24.63	14.42	251	15,060	8,810	1,748	1,022
W. F. B.	32	60.1	168	1.68	199	11,940	23.46	13.97	233	13,980	8,320	1,632	972
L. H. W.	27	60.0	179	1.76	184	11,040	21.70	12.34	219	13,140	7,470	1,530	869
H. B. L.	20	60.0	173	1.72	190	11,400	22.39	13.02	229	13,740	7,990	1,596	927
L. E. E.	31	59.8	175	1.73	204	12,240	24.06	13.93	245	14,700	8,490	1,707	987
F. M. M.	16	59.7	173	1.72	203	12,180	23.92	13.91	251	15,060	8,751	1,739	1,011
W. B. L.	29	59.3	164	1.65	165	9,900	19.43	11.77	211	12,660	7,670	1,451	879
T. H. Y.	22	59.2	169	1.68	190	11,400	22.39	13.32	231	13,860	8,250	1,605	955
D. R. S.	43	58.5	181	1.76	153	9,180	18.03	10.25	193	11,580	6,580	1,331	756
B. H.	39	58.2	178	1.73	166	9,960	19.56	11.32	200	12,000	6,930	1,393	806
D. J. M.	20	58.0	175	1.71	189	11,340	22.28	13.04	233	13,980	8,175	1,615	944
H. F. T.	32	57.8	179	1.73	165	9,900	19.43	11.24	192	11,520	6,660	1,348	779
E. T. W.	22	57.8	169	1.67	171	10,260	20.13	12.04	213	12,780	7,650	1,472	881
P. F. J.	20	57.2	167	1.64	193	11,580	22.74	13.87	232	13,920	8,480	1,616	985
L. D. A.	19	57.1	171	1.67	188	11,280	22.18	13.27	220	13,200	7,900	1,539	921
A. G. E.	26	57.0	169	1.65	195	11,700	22.99	13.93	216	12,960	7,850	1,531	928
R. I. C.	26	56.8	184	1.75	194	11,640	22.86	13.10	244	14,640	8,370	1,687	964
C. J. O.	27	56.7	160	1.58	...	......	.....	.....	218	13,080	8,275	1,524	965

TABLE III—CONTINUED

Name	Age	Weight in kgm.	Height in cm.	Body surface (DuBois)	A				B′			C′	
					CO_2 per min. (cc.)	CO_2 per hour (cc.)	CO_2 per hour (gms)	CO_2 per sq. m. per hour (gms.)	O_2 per min. (cc.)	O_2 per hr. (cc.)	O_2 per hr. per sq. m. (cc.)	Cals. total (24 hrs.)	Cals. per sq. m. (24 hrs.)
J. W. P........	30	56.5	172	1.67	203	12,180	23.92	14.33	243	14,580	8,730	1,697	1,017
W. W. C........	17	56.3	172	1.66	199	11,940	23.46	14.17	232	13,920	8,386	1,629	980
W. A. S........	21	56.3	169	1.64	190	11,400	22.39	13.65	223	13,380	8,160	1,562	952
J. C. C.........	22	56.1	173	1.67	179	10,740	21.10	12.64	219	13.140	7,870	1,522	912
Q. P. R........	41	52.2	164	1.56	158	9,480	18.63	11.93	193	11,580	7,420	1,341	859
O. N. A........	25	55.4	171	1.63	177	10,620	20.87	12.83	224	13,440	8,249	1,545	948
C. H. H........	19	55.1	169	1.63	173	10,380	20.39	12,52	203	12,180	7,475	1,421	872
I. A. F..........	24	54.9	156	1.53	190	11,400	22.39	14.63	232	13,920	9,090	1,612	1,053
V. G............	17	54.3	162	1.57	198	11,880	23.34	14.86	233	13,980	8,900	1,632	1,040
A. F. G.........	24	53.9	175	1,65	178	10,680	20.98	12.72	207	12,420	7,525	1,453	881
M. B............	27	53.6	160	1.54	170	10,200	20.03	13.02	210	12,600	8,182	1,455	945
L. E. A.........	30	52.2	174	1.62	191	11,460	22.52	13.89	219	13,140	8,115	1,541	952
B. M. K........	27	51.4	163	1.54	164	9,840	19.33	12.55	234	14,040	9,120	1,579	1,025
B. N. C.........	32	50.6	179	1.63	192	11,520	22.63	13.89	213	12,780	7,840	1,510	926
J. J. G..........	21	50.2	164	1.53	175	10,500	20.62	13.49	203	12,180	7,960	1,425	931
E. J. W.........	58	50.0	155	1.47	142	8,520	16.73	11.38	165	9,900	6,740	1,158	788
F. P............	17	49.3	161	1.50	188	11,280	22.18	14.75	229	13,740	9,160	1,591	1,061
V. E. H.........	21	49.3	163	1.51	157	9,420	18.50	12.26	198	11,880	7,870	1,365	904
T. M. C.........	35	48.5	165	1.52	156	9,360	18.39	12.10	185	11,100	7,300	1,292	850
J. H............	26	45.5	154	1.40	153	9,180	18.03	12.89	173	10,380	7,415	1,223	874

RESULTS BY DECADES—MEN

SECOND DECADE

Name	Age	A			B′			C′			Deviation of CO_2 from cals. in per ct.	Deviation of O_2 from cals. in per ct.
		Gms. CO_2 per sq. m. per hr.	Deviation of CO_2 from average	Per cent deviation of CO_2	Cc. O_2 per hour per sq. m.	Deviation of O_2 from average	Per cent deviation of O_2	Cals. per sq. m. 24 hours	Deviation of cals. from average	Per cent deviation of cals.		
H. W..................	19	15.82	+1.66	+11.7	8,914	+469	+ 5.6	1,053	+ 67	+ 6.8	+4.9	—1.2
M. H. K................	19	13.98	— .18	— 1.3	8,075	—370	— 4.4	949	— 37	— 3.8	+2.5	—0.6
F. M. M................	16	13.91	— .25	— 1.8	8,751	+306	+ 3.6	1,011	+ 25	+ 2.5	—4.3	+1.1
L. D. A.................	19	13.27	— .89	— 6.3	7,900	—545	— 6.5	921	— 65	— 6.6	+0.3	+0.1
W. W. C................	17	14.17	+ .01	+ .08	8,385	— 70	— .8	980	— 6	— 0.6	+0.7	—0.2
C. H. H.................	19	12.52	—1.64	—11.6	7,475	—970	—11.5	872	—114	—11.6	0.0	+0.1
V. G....................	17	14.86	+ .70	+ 4.9	8,900	+455	+ 5.4	1,040	+ 54	+ 5.5	—0.6	—0.1
F. P....................	17	14.75	+ .59	+ 4.2	9,160	+725	+ 8.6	1,061	+ 75	+ 7.6	—3.4	+1.0
Total..................	143	113.28			67,560			7,887				
Average..............	17.9	14.16			8,445			986				

THIRD DECADE

Name	Age	Gms. CO_2 per sq. m. per hr.	Deviation of CO_2 from average	Per cent deviation of CO_2	Cc. O_2 per hour per sq. m.	Deviation of O_2 from average	Per cent deviation of O_2	Cals. per sq. m. 24 hours	Deviation of cals. from average	Per cent deviation of cals.	Deviation of CO_2 from cals. in per ct.	Deviation of O_2 from cals. in per ct.
W. S....................	22	14.29	+1.16	+ 8.8	8,840	+ 764	+ 9.5	1,029	+101	+10.8	— 2.0	—1.3
O. F. M.................	24	12.46	— .67	— 5.1	8,030	— 46	— 0.6	922	— 16	— 1.7	— 3.4	+1.1
J. H. R.................	23	13.84	+0.71	+ 5.4	8,174	+ 354	+ 4.4	956	+ 18	+ 1.9	+ 3.5	+2.5
D. H. W................	22	14.03	+0.90	+ 6.9	8,475	+ 399	+ 4.9	987	+ 49	+ 5.2	+ 1.7	—0.3
E. G....................	20	15.09	+1.96	+14.9	8,970	+ 864	+10.7	1,053	+115	+12.3	+ 2.6	—1.6
W. A. M................	23	12.52	—0.61	— 4.6	7,860	— 216	— 2.7	907	— 31	— 3.3	— 1.3	+0.6
D. R. M................	28	12.95	—0.18	— 1.4	8,400	+ 324	+ 4.0	962	+ 24	+ 2.6	— 4.0	+1.4
M. B....................	20	13.52	+0.39	+ 3.0	8,130	+ 54	+ 0.7	947	+ 9	+ 1.0	+ 2.0	—0.3
J. F. M.................	20	13.73	+0.60	+ 4.6	8,280	+ 204	+ 2.5	963	+ 25	+ 2.7	+ 1.9	—0.2
W. J. T.................	22	12.43	—0.70	— 5.3	7,875	— 201	— 2.5	908	— 21	— 2.2	— 3.1	—0.3
F. G. R.................	20	14.87	+1.74	+13.3	8,300	+ 324	+ 4.0	992	+ 54	+ 5.8	+ 7.5	—1.8
C. D. R.................	22	14.92	+1.79	+13.6	8,620	+ 544	+ 6.7	1,015	+ 77	+ 8.2	+ 5.4	—1.5
H. R. W................	24	13.85	+0.72	+ 5.5	8,380	+ 304	+ 3.8	974	+ 36	+ 3.8	+ 1.7	0.0
J. P. C.................	23	11.85	—1.71	—13.0	7,070	—1,006	—12.5	825	—113	—12.0	— 1.0	—0.5
A. O. G.................	25	11.92	—1.21	— 9.2	8,470	+ 394	+ 4.9	956	+ 18	+ 1.9	—11.1	+3.0
H. W. E................	23	12.23	—0.90	— 6.9	7,275	— 801	— 9.9	852	— 86	— 9.2	+ 2.3	—0.7
P. D. F.................	23	13.83	+0.70	+ 5.3	8,320	+ 244	+ 3.0	968	+ 30	+ 3.2	+ 2.1	—0.2
C. B. S.................	26	12.63	—0.53	— 4.0	7,440	— 636	— 7.9	899	— 39	— 4.2	+ 0.2	—3.7
J. H. H.................	25	12.84	—0.29	— 2.2	7,752	— 324	— 4.0	903	— 35	— 3.7	+ 1.5	—0.3
B. A. W................	26	14.84	+1.71	+13.0	9,231	+1,149	+14.2	1,069	+131	+14.0	— 1.0	+0.2
H. H. A................	26	12.32	—0.81	— 6.2	7,675	— 401	— 5.0	889	— 49	— 5.2	— 1.0	+0.2
J. R....................	27	12.82	—0.31	— 2.4	7,815	— 261	— 3.2	907	— 31	— 3.3	+ 0.9	+0.1
M. A. M................	29	13.42	+0.29	+ 2.2	8,025	— 51	— 0.6	937	— 1	— 0.1	+ 2.3	—0.5
F. P. R.................	22	12.08	—1.05	— 8.0	7,480	— 596	— 7.4	867	— 71	— 7.6	— 0.4	+0.2
J. J. C..................	27	12.49	—0.64	— 4.9	7,610	— 466	— 5.8	886	— 52	— 5.5	+ 0.6	—0.3

TABLE III—Continued

Name	Age	A			B′			C′				
		Gms. CO_2 per sq. m. per hr.	Deviation of CO_2 from average	Per cent deviation of CO_2	Cc. O_2 per hour per sq. m.	Deviation of O_2 from average	Per cent deviation of O_2	Cals. per sq. m. 24 hours	Deviation of cals. from average	Per cent deviation of cals.	Deviation of CO_2 from cals. in per ct.	Deviation of O_2 from cals. in per ct.
E. H. T.	25	11.64	—1.49	—11.3	7,440	— 646	— 8.0	857	— 81	— 8.7	— 2.6	+0.7
P. M.	22	12.62	—0.51	— 3.9	8,225	+ 149	+ 1.8	944	+ 6	+ 0.6	— 4.5	+1.2
M. J. S.	24	13.22	+0.09	+ 0.7	8,170	+ 94	+ 1.2	947	+ 9	+ 1.0	— 0.3	+0.2
M. Y. B.	20	13.97	+0.84	+ 6.4	8,160	+ 84	+ 1.0	958	+ 30	+ 3.2	+ 3.2	—2.2
R. D. S.	21	13.88	+0.65	+ 5.0	7,860	— 216	— 2.7	930	— 8	— 0.9	+ 5.9	—1.8
R. G.	23	13.08	—0.05	— 0.4	7,780	— 296	— 3.7	908	— 30	— 3.2	+ 2.8	—0.5
V. R. B.	27	12.07	—1.06	— 8.1	7,540	— 536	— 6.6	872	— 66	— 7.1	— 1.0	+0.5
W. F. M.	21	14.48	+1.35	+10.3	8,640	+ 566	+ 7.0	1,009	+ 71	+ 7.6	+ 2.7	—0.6
H. H. A.	22	12.57	—0.56	— 4.3	7,610	— 466	— 5.8	886	— 52	— 5.6	+ 1.3	—0.2
H. C. B.	27	13.03	—0.10	— 0.7	8,240	+ 164	+ 2.0	950	+ 12	+ 1.3	— 2.1	+0.7
S. A. R.	23	12.35	—0.78	— 7.8	7,509	— 561	— 7.0	873	— 65	— 6.9	— 0.9	—0.1
W. G. J.	21	14.23	+1.10	+ 8.4	8,621	+ 539	+ 6.7	1,003	+ 65	+ 6.9	+ 1.5	—0.2
H. L. H.	26	13.71	+0.58	+ 4.4	8,560	+ 384	+ 4.8	985	+ 47	+ 5.0	— 0.6	—0.2
J. E. F.	21	13.87	+0.74	+ 5.6	7,990	— 86	— 1.1	940	+ 2	+ 0.2	+ 5.4	—1.3
J. K. M.	24	12.42	—0.71	— 5.4	7,730	— 346	— 4.3	895	— 43	— 4.6	— 0.8	+0.3
H. B. R.	25	12.13	—1.00	— 7.6	7,640	— 436	— 5.4	885	— 53	— 5.7	— 1.9	+0.3
J. B. T.	20	14.42	+1.29	+ 9.8	8,810	+ 734	+ 9.1	1,022	+ 84	+ 9.0	+ 0.8	+0.1
L. H. W.	27	12.34	—0.79	— 6.0	7,470	— 606	— 7.5	869	— 69	— 7.4	+ 1.4	—0.1
H. B. L.	20	13.02	—0.11	— 0.8	7,990	— 86	— 1.1	922	— 16	— 1.7	+ 0.9	+0.6
W. B. L.	29	11.77	+0.20	+ 1.5	7,670	— 406	— 5.0	879	— 59	— 6.3	+ 7.8	+1.3
H. Y.	22	13.32	+0.19	+ 1.4	8,250	+ 174	+ 2.2	955	+ 17	+ 1.8	— 0.4	+0.4
D. J. M.	20	13.04	—0.09	— 0.7	8,175	+ 99	+ 1.2	944	+ 6	+ .6	— 1.3	+0.6
E. T. W.	22	12.04	—1.09	— 8.3	7,650	— 426	— 5.3	881	— 77	— 8.2	— 0.1	+2.9
P. F. J.	20	13.87	+0.74	+ 5.6	8,480	+ 404	+ 5.0	985	+ 47	+ 5.0	+ 0.6	0.0
A. G. E.	26	13.93	+0.80	+ 6.1	7,850	— 226	— 2.8	928	— 10	— 1.1	+ 7.2	—1.7
R. I. C.	26	13.10	—0.03	— 0.2	8,370	+ 294	+ 3.6	964	+ 26	+ 2.8	— 3.0	+0.8
C. J. O.	27	.....	......	......	8,275	+ 199	+ 2.5	965	+ 27	+ 2.9	......	......
W. A. S.	21	13.65	+0.52	+ 4.0	8,160	+ 84	+ 1.0	952	+ 14	+ 1.5	+ 2.5	—0.5
J. C. C.	22	12.64	—0.49	— 3.7	7,870	— 206	— 2.6	912	— 26	— 2.8	— 0.9	+0.2
O. N. A.	25	12.83	—0.30	— 2.3	8,249	+ 173	+ 2.1	948	+ 10	+ 1.1	— 3.4	+1.0
I. A. F.	24	14.63	+1.50	+11.4	9,090	+1,014	+12.6	1,053	+115	+12.3	— 0.9	+0.3
A. F. G.	24	12.72	—0.41	— 3.1	7,525	— 551	— 6.8	881	— 57	— 6.1	+ 3.0	—0.7
M. B.	27	13.02	—0.11	— 0.8	8,182	+ 99	+ 1.2	945	+ 7	+ 0.7	— 1.5	+0.5
B. M. K.	27	12.55	—0.58	— 4.4	9,120	+1,044	+12.9	1,025	+ 87	+ 9.3	—13.7	+3.6
J. J. G.	21	13.49	+0.36	+ 2.7	7,960	— 116	— 1.4	931	— 7	— 0.7	+ 3.4	—0.7
V. E. H.	21	12.26	—0.87	— 6.6	7,870	— 206	— 2.6	904	— 34	— 3.6	— 3.0	+1.0
J. H.	26	12.89	—0.24	— 1.8	7,415	— 661	— 8.2	874	— 64	— 6.8	+ 5.0	—1.4
Total	1461	802.57			500,742			58,163				
Average	23.6	13.13			8,076			938				
FOURTH DECADE												
Prof. C.	36	12.17	— .72	— 5.6	7,370	— 345	— 4.5	857	— 43	— 4.8	— 0.8	+0.3
F. E. M.	38	13.53	+ .64	+ 5.0	7,980	+ 265	+ 3.4	932	+ 32	+ 3.6	+ 1.4	—0.2
F. A. R.	32	13.43	+ .54	+ 4.2	8,135	+ 420	+ 5.4	948	+ 48	+ 5.3	— 1.1	+0.1
N. K. W.	35	13.06	+ .17	+ 1.3	7,425	— 290	— 3.8	875	— 25	— 2.8	+ 4.1	—1.0
E. P. C.	35	10.88	—2.11	—16.4	7,001	— 714	— 9.2	805	— 95	—10.6	— 5.8	+1.4
W. F. B.	32	13.97	+1.08	+ 8.4	8,320	+ 605	+ 7.8	972	+ 72	+ 8.0	+ 0.4	—0.2
L. E. E.	31	13.93	+1.04	+ 8.1	8,490	+ 775	+10.0	987	+ 87	+ 9.7	— 1.6	+0.3
B. H.	39	11.32	—1.57	—12.2	6,930	— 785	—10.2	806	— 94	— 9.6	— 2.6	—0.6
H. F. T.	32	11.24	—1.65	—12.8	6,660	—1,055	—13.7	779	—121	—13.4	+ 0.6	—0.3
J. W. P.	30	14.33	+1.44	+11.2	8,730	+1,015	+13.2	1,017	+117	+13.0	— 1.8	+0.2
L. E. A.	30	13.89	+1.00	+ 7.8	8,115	+ 400	+ 5.2	952	+ 52	+ 5.8	+ 2.0	—0.6
B. N. C.	32	13.89	+1.00	+ 7.8	7,840	+ 125	+ 1.6	926	+ 26	+ 2.9	+ 4.9	—1.3
T. M. C.	35	12.10	—0.79	— 6.1	7,300	— 415	— 5.4	850	— 50	— 5.6	— 0.5	+0.2
Total	437	167.64			100,296			11,706				
Average	33.6	12.89			7,715			900				
FIFTH DECADE												
F. G. B.	41	12.47	+ .59	+ 5.0	7,551	+ 318	+ 4.3	879	+ 31	+ 3.6	+ 1.4	+0.7
A. L.	40	13.17	+1.01	+ 8.5	7,890	+ 488	+ 6.6	922	+ 66	+ 7.7	+ 0.8	—1.1
Dr. S.	43	10.25	—1.63	—13.7	6,580	— 822	—11.1	756	—100	—11.7	— 2.0	+0.6
Dr. P. R.	41	11.93	+ .05	+ 0.4	7,420	+ 18	+ 0.2	859	+ 3	+ 0.4	0.0	—0.2
Total	165	47.54			29,610			3,424				
Average	41.2	11.88			7,402			856				
SIXTH DECADE												
E. J. W.	58	11.38			6,740			788				
SEVENTH DECADE												
H. F.	63	11.19			7,449			851				

TABLE III—CONTINUED

NORMAL WOMEN

Name	Age	Weight in kgm.	Height in cm.	Body surface (DuBois)	A				B′			C′	
					CO_2 per min. (cc.)	CO_2 per hr. (cc.)	CO_2 per hour (gms.)	CO_2 per sq. m. per hour (gms.)	O_2 per min. (cc.)	O_2 per hr. (cc.)	O_2 per hour per sq. m. (cc.)	Calculated cals. (total 24 hours)	Cals. per sq. m. (24 hrs.)
Dr. M. D.......	44	93.6	165	2.0	202	12,120	23.79	11.89	256	15,360	7,680	1,765	882.5
Miss O. A......	21	90.2	164	1.96	203	12,180	23.94	12.22	254	15,240	7,786	1,756	896.0
Miss H. H......	21	88.3	161	1.92	190	11,400	22.39	11.67	228	13,680	7,120	1,584	825.1
Mrs. H. D......	42	80.1	157	1.81	184	11,040	21.69	11.99	233	13,980	7,725	1,606	887.5
Miss C. Z......	39	67.2	170	1.78	176	10,560	20.75	11.65	220	13,200	7,410	1,521	854.9
Miss S.........	27	65.5	171	1.77	178	10,680	20.98	11.85	202	12,120	6,840	1,426	805.1
Miss C. H......	25	63.4	166	1.71	156	9,360	18.39	10.75	207	12,420	7,260	1,413	827.0
Miss A. K......	23	63.2	171	1.75	165	9,900	19.44	11.13	202	12,120	6,925	1,402	801.0
Miss A. G......	21	63.0	161	1.67	153	9,180	18.04	10.82	192	11,520	6,895	1,324	792.5
Miss V. A......	21	62.9	168	1.72	167	10,020	19.68	11.44	188	11,280	6,552	1,324	770.0
Miss C.........	22	61.9	168	1.70	177	10,620	20.83	12.26	203	12,180	7,165	1,427	839.0
Miss K. K......	21	61.5	154	1.59	....	......	......	......	238	14,280	8,975	1,666	1,048
Dr. A. B.......	32	60.3	163	1.65	197	11,820	23.21	14.08	207	12,420	7,525	1,486	901
Miss L. G......	38	59.5	159	1.61	134	8,040	15.83	9.84	173	10,380	6,448	1,187	737
Miss B. W......	22	59.4	162	1.63	180	10,800	21.20	13.02	223	13,380	8,210	1,546	947
Miss L. O......	23	59.3	169	1.68	176	10,560	20.75	12.35	207	12,420	7,400	1.448	862
Miss M. W......	25	58.6	167	1.65	168	10,080	19.79	11.99	206	13,360	7,485	1,429	865
Miss M. P......	28	58.1	168	1.66	170	10,200	20.03	12.07	222	13,320	8,020	1,518	914
Mrs. E. B......	53	58.0	163	1.63	171	10,260	20.16	12.36	202	12,120	7,435	1,415	867
Miss M. M......	18	57.9	164	1.63	191	11,460	22.50	13.81	207	12,420	7,615	1,475	904
Miss E. P......	23	57.7	175	1.70	166	9,960	19.60	11.53	207	12,420	7,310	1,430	841
Miss L. K......	22	56.8	166	1.63	153	9,180	18.04	11.07	199	11,940	7,325	1,365	838
Miss E. A......	15	56.8	157	1.56	205	12,300	24.18	15.49	231	13,860	8,880	1,630	1,044
Miss A. M......	20	56.8	152	1.52	155	9,300	18.24	12.01	192	11,520	7,575	1,329	873
Miss J. C......	22	55.1	162	1.58	157	9,420	18.52	11.72	198	11,880	7,520	1,363	862
Miss G. L......	21	55.0(?)	166	1.61	173	10,380	20.39	12.66	214	12,840	7,970	1,480	919
Mrs. D. C......	36	54.9	153	1.51	160	9,600	18.86	12.49	182	10,920	7,230	1,278	845
Miss M. T......	20	54.5	164	1.58	147	8,820	17.35	10.98	196	11,760	7,440	1,359	859
Miss F. K......	18	54.1	164	1.58	157	9,420	18.52	11.72	179	10,740	6,800	1,262	798
Miss J. N. B...	26	53.8	160	1.55	134	8,040	15.81	10.19	178	10,680	6,885	1,215	784
Miss L. T......	31	53.6	155	1.51	152	9,120	17.95	11.89	178	10,680	7,070	1,247	825
Miss F. E......	22	53.1	162	1.56	161	9,660	19.00	12.18	202	12,120	7,770	1,391	892
Miss L.........	25	52.4	168	1.58	162	9,720	19.12	12.09	188	11,280	7,140	1,321	836
Miss A. D......	37	52.3	166	1.57	159	9,540	18.78	11.97	196	11,760	7,485	1,355	863
Miss B.........	21	52.2	158	1.52	173	10,380	20.39	13.42	202	12,120	7,970	1,409	926
Miss R. M......	16	52.1	162	1.54	159	9,540	18.78	12.21	195	11,700	7,590	1,353	879
Miss L. J......	24	51.8	159	1.52	142	8,520	16.77	11.03	179	10,740	7,070	1,235	812
Mrs. A.........	29	51.6	163	1.54	166	9,960	19.60	12.73	205	12,300	7,980	1.421	922
Miss J. M......	16	51.4	158	1.50	....	......	......	......	220	13,200	8,795	1,541	1,028
Miss J. B......	27	51.1	163	1.53	146	8,760	17.23	11.27	183	10,980	7,175	1,265	827
Miss R. A......	21	50.8	155	1.47	161	9,660	19.00	12.92	184	11,040	7,510	1,296	881
Miss M. C......	16	50.6	162	1.52	145	8,700	17.11	11.26	185	11,100	7,300	1,273	838
Miss E. C......	25	50.5	164	1.53	155	9,300	18.24	11.93	192	11,520	7,525	1,327	867
Miss I. B......	18	50.1	166	1.55	160	9,600	18.86	12.17	173	10,380	6,700	1,235	797
Miss E. S......	25	50.0	164	1.53	154	9,240	18.19	11.89	195	11,700	7,647	1,345	879
Miss C. B......	24	49.8	162	1.51	168	10,080	19.79	13.10	205	12,300	8,140	1,408	932
Miss G. V......	22	49.7	160	1.50	128	7,680	15.12	10.09	166	9,960	6,645	1,139	759
Miss D. W......	19	49.4	160	1.50	153	9,180	18.03	12.03	187	11,220	7,475	1,300	866
Miss M. H......	27	49.1	151	1.43	135	8,100	15.95	11.15	171	10,260	7,170	1,178	823
Miss C. L......	21	49.1	151	1.43	153	9,180	18.03	12.62	195	11,700	8,175	1,341	938
Miss C. E......	74	48.9	164	1.52	122	7,320	14.40	9.47	160	9,600	6,320	1,095	721
Miss V. M......	24	48.9	162	1.50	149	8,940	17.59	11.72	198	11,880	7,915	1,351	902
Miss M. S......	24	48.5	159	1.48	167	10,020	19.68	13.29	216	12,960	8,751	1,480	1,000
Miss G. F......	27	48.5	155	1.45	140	8,400	16.53	11.41	180	10,800	7,445	1,233	852
Miss K. M......	22	48.2	161	1.49	161	9,660	19.00	12.75	184	11,040	7,415	1,294	868
Miss L. B......	27	47.0	167	1.50	136	8,160	16.05	10.71	169	10,140	6,770	1,168	778
Miss E. T......	22	46.7	164	1.48	151	9,060	17.79	12.02	195	11,700	7,900	1,336	902
Miss H. T......	25	45.0	159	1.43	156	9,360	18.40	12.87	203	12,180	8,515	1,393	974
Miss R. W......	21	45.0(?)	153	1.39	141	8,460	16.65	11.98	186	11,160	8,025	1,273	916
Mrs. A. L......	29	44.9	159	1.43	147	8,820	17.35	12.13	184	11,040	7,720	1,272	889
Miss M. J......	27	44.8	157	1.42	137	8,220	16.18	11.40	172	10,320	7,270	1,189	837
Miss J.........	24	43.0	159	1.41	139	8,340	16.41	11.64	165	9,900	7,020	1,158	821
Miss A. C......	38	42.6	165	1.44	138	8,280	16.30	11.32	168	10,080	6,995	1,168	811
Miss D. A......	19	41.5	154	1.35	136	8,160	16.05	11.89	176	10,560	7,820	1,207	893
Miss E. W......	24	40.5	157	1.35	153	9,180	18.03	13.37	183	10,980	8,130	1,275	944
Miss J. T......	36	40.0	168	1.42	139	8,340	16.41	11.57	186	11,160	7,855	1,269	892
Mrs. S. C......	52	37.4	155	1.29	122	7,320	14.39	11.15	140	8,400	6,515	1,013	786
Mrs. A. A......	43	35.6	170	1.35	119	7,140	14.04	10.41	169	10,140	7,515	1,141	844

TABLE III—Continued

Results by Decades—Women

Name	Age	A: Gms. CO_2 per sq. m. per hr.	A: Deviation of CO_2 from average	A: Per cent deviation of CO_2	B′: Cc. O_2 per hour per sq. m.	B′: Deviation of O_2 from average	B′: Per cent deviation of O_2	C′: Cals. per sq. m. (24 hours)	C′: Deviation of cals. from average	C′: Per cent deviation of cals.	Deviation of CO_2 from cals. in per cent	Deviation of O_2 from cals. in per cent
SECOND DECADE												
Miss M. M.	18	13.81	+1.24	+ 9.9	7,615	+ 93	— 1.2	904	+ 27	+ 3.1	+6.8	—4.3
Miss E. A.	15	15.49	+2.92	+23.2	8,880	+1,358	+18.00	1,044	+167	+19.1	+4.1	—1.1
Miss F. K.	18	11.72	—0.85	— 6.8	6,800	— 722	— 9.6	798	— 79	— 9.0	+2.2	—0.6
Miss R. M.	16	12.21	—0.36	— 2.9	7,590	+ 68	— 0.9	879	+ 2	+ 0.2	—3.1	—1.1
Miss J. M.	16	......	......	......	(8,795)	......	......	(1,028)	......	......	......	......
Miss M. C.	16	11.26	—1.31	—10.4	7,300	— 222	— 3.0	838	— 39	— 4.4	—6.0	+1.4
Miss I. B.	18	12.17	—0.40	— 3.2	6,700	— 822	—10.9	797	— 80	— 9.0	+5.8	—1.9
Miss D. W.	19	12.03	—0.54	— 4.3	7,475	— 47	— 0·6	866	— 11	— 1.3	—3.0	+0.7
Miss D. A.	19	11.89	—0.68	— 5.4	7,820	+ 298	+ 4.0	893	+ 16	+ 1.8	—7.2	+2.2
Total	155	100.58			60,180			7,019				
Average	17.2	12.57			7,522			877				
THIRD DECADE												
Miss O. A.	21	12.22	+ 33	+ 2.8	7,786	+ 275	+ 3.7	896	+ 30	+ 3.5	—0.7	+0.2
Miss H. H.	21	11.67	— 22	— 1.9	7,120	— 391	— 5.2	825	— 41	— 4.7	+2.6	—0.5
Miss S.	27	11.85	— 4	— 0.3	6,840	— 671	— 9.0	805	— 61	— 7.0	+6.7	—2.0
Miss C. H.	25	10.75	—114	— 9.6	7,260	— 251	— 3.4	827	— 39	— 4.5	—5.1	+1.1
Miss A. K.	23	11.13	— 76	— 6.4	6,925	— 586	— 7.8	801	— 65	— 7.5	+1.1	—0.3
Miss A. G.	21	10.82	—107	— 9.0	6,895	— 616	— 8.2	792	— 74	— 8.5	—0.5	+0.3
Miss V. A.	21	11.44	— 45	— 3.8	6,552	— 959	—12.8	770	— 96	—11.1	+7.3	—1.7
Miss C.	22	12.26	+ 37	+ 3.1	7,165	— 346	— 4.6	839	— 27	— 3.1	+6.2	—1.5
Miss B. W.	22	13.02	+113	+ 9.5	8,210	+ 699	+ 9.3	947	+ 81	+ 9.4	+0.1	—0.1
Miss L. B.	23	12.35	+ 46	+ 3.9	7,400	— 111	— 1.5	862	— 4	— 0.5	+4.4	—1.0
Miss M. W.	25	11.99	+ 10	+ 0.8	7,485	— 26	— 0.35	865	— 1	— 0.1	+0.9	—2.5
Miss M. P.	28	12.07	+ 18	+ 1.5	8,020	+ 509	+ 6.8	914	+ 48	+ 5.6	—4.1	+1.2
Miss E. P.	23	11.53	— 36	— 3.0	7,310	— 201	— 2.7	841	— 25	— 2.9	—0.1	+0.2
Miss L. K.	22	11.07	— 82	— 6.9	7,325	— 186	— 2.5	838	— 28	— 3.2	—3.7	+0.7
Miss A. M.	20	12.01	+ 12	+ 1.1	7,575	+ 64	+ 0.9	873	+ 7	+ 0.8	+0.3	+0.1
Miss J. C.	22	11.72	— 17	— 1.4	7,520	+ 9	+ 0.1	862	— 4	— 0.5	—0.9	+0.6
Miss G. L.	21	12.66	+ 77	+ 6.5	7,970	+ 459	+ 6.1	919	+ 53	+ 6.1	+0.4	±0.0
Miss M. T.	20	10.98	— 91	— 7.7	7,440	— 71	— 0.9	859	— 7	— 0.8	—6.9	—0.1
Miss J. N. B.	26	10.19	—170	—14.3	6,885	— 626	— 8.4	784	— 82	— 9.5	—4.8	+1.1
Miss F. E.	22	12.18	+ 29	+ 2.5	7,770	+ 259	+ 3.6	892	+ 26	+ 3.0	—0.5	+0.6
Miss L.	25	12.09	+ 20	+ 1.7	7,140	— 371	— 5.0	836	— 30	— 3.5	+5.2	—1.5
Miss B.	21	13.42	+153	+12.9	7,970	+ 459	+ 6.1	926	+ 60	+ 7.0	+5.9	—0.9
Miss L. J.	24	11.03	— 86	— 7.2	7,070	— 441	— 5.9	812	— 54	— 6.2	—1.0	+0.3
Mrs. A.	29	12.73	+ 84	+ 7.1	7,980	+ 469	+ 6.3	922	+ 56	+ 6.5	+0.6	—0.2
Miss J. B.	27	11.27	— 62	— 5.2	7,175	— 336	— 4.5	827	— 39	— 4.5	—0.7	±0.0
Miss R. A.	21	12.92	+103	+ 8.7	7,510	— 1	± 0.0	881	+ 15	+ 1.7	+7.0	—1.7
Miss E. C.	25	11.93	+ 4	+ 0.3	7,525	+ 14	+ 0.2	867	+ 1	+ 0.1	+0.2	+0.1
Miss E. S.	25	11.89	......	......	7,647	+ 136	— 7.0	879	+ 13	+ 1.5	—1.5	—8.5
Miss C. B.	24	13.10	+121	+10.2	8,140	+ 629	+ 8.4	932	+ 66	+ 7.6	+2.6	+0.8
Miss G. J.	22	10.09	—180	—15.1	6,645	— 866	—11.5	759	—107	—12.4	—2.7	+0.9
Miss M. H.	27	11.15	— 74	— 6.2	7,170	— 341	— 4.6	823	— 43	— 5.0	—1.2	+0.4
Miss C. L.	21	12.62	+ 73	+ 6.1	8,175	+ 664	+ 8.9	938	+ 72	+ 8.3	—2.2	+0.6
Miss V. M.	24	11.72	— 17	— 1.4	7,915	+ 404	+ 5.4	902	+ 36	+ 4.2	—5.6	+1.2
Miss M. S.	24	13.29	+140	+11.8	8,751	+1,240	+16.6	1,000	+134	+15.5	—3.7	+1.1
Miss G. F.	27	11.41	— 48	— 4.0	7,445	— 66	— 0.9	852	— 14	— 1.6	—2.4	+0.7
Miss K. M.	22	12.75	+ 86	+ 7.2	7,415	— 96	— 1.3	868	+ 2	+ 0.2	+7.0	—1.5
Miss L. B.	27	10.71	—118	— 9.9	6,770	— 741	— 9.9	778	— 88	—10.2	+0.3	+0.3
Miss E. T.	22	12.02	+ 13	+ 1.1	7,900	+ 389	+ 5.2	902	+ 36	+ 4.2	—3.1	+1.0
Miss H. T.	25	12.87	+ 98	+ 8.3	8,515	+1,004	+13.4	974	+108	+15.5	—4.2	+0.9
Miss R. W.	21	11.98	+ 9	+ 0.8	8,025	+ 514	+ 6.9	916	+ 50	+ 5.8	—5.0	+1.1
Mrs A. L.	29	12.13	+ 24	+ 2.0	7,720	+ 209	+ 2.8	889	+ 23	+ 2.7	—0.7	+0.1
Miss M. J.	27	11.40	— 49	— 4.1	7,270	— 241	— 3.2	837	— 29	— 3.4	—0.7	+0.2
Miss J.	24	11.64	— 25	— 2.1	7,020	— 491	— 6.6	821	— 45	— 5.2	+3.1	—1.4
Miss E. W.	24	13.37	+148	+12.5	8,130	+ 619	+ 8.3	944	+ 78	+ 9.0	+3.5	—0.7
Total	1,042	52,344			330,476			38,096				
Average	23.7	11.89			7,511			866				
FOURTH DECADE												
Miss C. Z.	39	11.65	—0.20	— 1.7	7,410	+158	+ 2.2	855	+ 14	+ 1.7	— 3.4	+0.5
Dr. A. B.	32	14.08	+2.23	+18.8	7,525	+273	+ 3.8	901	+ 60	+ 7.1	+11.7	+3.3
Miss L. G.	38	9.84	—2.01	—17.0	6,448	—804	—11.1	737	—104	—12.4	— 4.6	+1.3
Mrs. D. C.	36	12.49	+0.64	+ 5.4	7,230	— 22	— 0.3	845	+ 4	+ 0.5	+ 4.9	—0.8
Miss L. T.	31	11.89	+0.04	+ 0.3	7,070	—182	— 2.5	825	— 16	— 1.9	+ 2.2	—0.6
Miss A. D.	37	11.97	+0.12	+ 1.0	7,485	+233	+ 3.2	863	+ 22	+ 2.6	— 1.6	+0.6
Miss A. C.	38	11.32	—0.53	— 4.5	6,995	—257	— 3.5	811	— 30	— 3.6	— 0.9	+0.1
Miss J. T.	36	11.57	—0.28	— 2.4	7,855	+603	+ 8.3	892	+ 51	+ 6.1	— 8.5	+2.2
Total	287	94.81			58,018			6,729				
Average	35.9	11.85			7,252			841				

TABLE III—CONTINUED

Name	Age	A			B′			C′			Deviation of CO_2 from cals. in per ct.	Deviation of O_2 from cals. in per ct.
		Gms. CO_2 per sq. m. per hour	Deviation of CO_2 from average	Per cent deviation of CO_2	Cc. O_2 per hour per sq. m.	Deviation of O_2 from average	Per cent deviation of O_2	Cals. per sq. m. (24 hours)	Deviation of cals. from average	Per cent deviation of cals.		
FIFTH DECADE												
Dr. M. D.	44	11.89	+0.46	+4.0	7,680	+ 40	+0.5	883	+11	+1.3	+2.7	—0.8
Mrs. H. D.	42	11.99	+0.56	+4.9	7,725	+ 85	+1.1	888	+16	+1.8	+3.1	—0.7
Mrs. A. A.	43	10.41	—1.02	—8.9	7,515	—125	—1.6	844	—28	—3.2	—5.7	+1.6
Total	129	34.29			22,920			2,615				
Average	43	11.43			7,640			872				
SIXTH DECADE												
Mrs. E. B.	53	12.36	+0.60	+5.1	7,435	+460	+6.6	867	+41	+5.0	+0.1	+1.6
Mrs. S. C.	52	11.15	—0.61	—5.1	6,515	—460	—6.6	786	—40	—5.0	—0.1	—1.6
Total	105	23.51			13,950			1,653				
Average	52.5	11.76			6,975			826				
SEVENTH DECADE												
Miss C. E.	74	9.47	......	.....	6,320	.....	.....	721	....	.....	.....	.....

CO_2 elimination in a large number of normal individuals. This scale is shown later in this paper and is seen to correspond closely with the scale constructed by the writer. The only significant discrepancies between the two scales occur in the upper decades. It happens, however, that the bulk of subjects studied by Benedict and his associates fell by age into the lower decades, making the figures for these decades more significant. It is probable that the scale computed by the writer, which follows the curve constructed by Aub and DuBois,[8] is somewhat nearer the actual average for the upper decades.

We have not attempted to draw conclusions from Table III as to the correlation of the O_2 and CO_2 figures, respectively, with the calories, inasmuch as the calories are calculated calories (indirect method) and not measured calories (direct method). It is seen at a glance, however, that the calorie figures follow the O_2 figures more closely than they follow the CO_2 figures. It is, therefore, obvious that the practical result of the customary method of deriving calories from O_2 and CO_2 figures is that the O_2 figure is more influential in determining the resultant calorie figures than the CO_2 figures are.

To make our figures of more value we requested Dr. Pearl to make a statistical review of them, and are indebted to him for the following:

MEMORANDUM RE CALORIMETRY

The point at issue is whether CO_2 output may be used as a basis of calorie calculation with as much validity as attached to the use of O_2 intake for the same purpose.

[8] Aub and DuBois: The Basal Metabolism of Old Men. Arch. Int. Med., 1917, XVIII, 823.

To test the point adequately it is necessary to determine: (a) whether on the same material the variation of the CO_2 figures significantly differs from the variation of the O_2 figures, and (b) whether on the same material there is any significant difference between O_2 intake and CO_2 output in respect of the degree of their correlation with calories per square meter.

The significant figures in question (a) will be coefficients of variation rather than standard deviations, because of different units involved, and because it is *relative* rather than absolute variability in which we are interested.

Table 1 furnishes the needed constants for each of the four series of original data. A standard deviation is denoted by σ and a coefficient of variation by V.

Let subscript letters have the following significance:

$A = CO_2$ per $(M)^2$ per hour in grams.
$B = O_2$ per $(M)^2$ per hour in grams.
$C =$ Calories per $(M)^2$ per hour.
$B' = O_2$ per $(M)^2$ per hour in c. c.
$C' =$ Calories per $(M)^2$ per 24 hours.

The significant comparisons in respect of variability are:

Direct calorimetry (10 cases)

$V_A - V_B = 7.22 - 12.93 = -5.71 \pm 2.26$ Diff/P.E.$_{Diff} = 2.5$.

Direct calorimetry (17 cases)

$V_A - V_B = 10.50 - 10.40 = + .10 \pm 1.73$ Diff/P.E.$_{Diff} = .06$.

Normal men

$V_A - V_B = 7.94 - 7.07 = + .87 \pm .54$ Diff/P.E.$_{Diff} = 1.6$.

Normal women

$V_A - V_B = 8.37 - 7.06 = +1.31 \pm .65$ Diff/P.E.$_{Diff} = 2.0$.

From these figures we conclude that:

1. There is no significant difference in relative variability, in any of the four series, between O_2 intake and CO_2 output.

TABLE 1

	CO_2 per sq. m. per hr. (Gms.) = A	O_2 per sq. m. per hr. (Gms.) = B	Cals. per sq. m. per hr. = C	O_2 per sq. m. per hr. (cc.) = B′	Cals. per sq. m. 24 hrs. = C′
Direct calorimetry (10 cases): mean	+14.08± .22	+12.20± .34	+44.87± .77	....	....
Direct calorimetry (10 cases): σ	1.02± .15	1.58± .24	3.61± .54	....	....
Direct calorimetry (10 cases): V	7.22±1.09	12.93±1.98	8.04±1.22	....	....
Direct calorimetry (17 cases): mean	13.21± .23	11.95± .20	38.07± .74	....	....
Direct calorimetry (17 cases): σ	1.39± .16	1.24± .14	4.53± .52	....	....
Direct calorimetry (17 cases): V	10.50±1.23	10.40±1.22	11.89±1.40	....	....
Indirect calorimetry (17 cases): mean	*	*	+38.47± .58	....	....
Indirect calorimetry (17 cases): σ	*	*	3.56± .41	....	....
Indirect calorimetry (17 cases): V	—	—	9.25±1.08	....	....
Normal men: mean	+13.10± .08	....	....	+7995.45±40.65	931.53±4.72
Normal men: σ	1.04± .05	....	....	565.42±28.75	65.71±3.34
Normal men: V	7.94± .41	....	....	7.07± .36	7.05± .36
Normal women: mean	+11.90± .08	....	....	7454.55±43.69	861.36±4.72
Normal women: σ	1.00± .06	....	....	526.28±30.90	56.89±3.34
Normal women: V	8.37± .51	....	....	7.06.00± .42	6.61± .39

* Omitted because identical with immediately preceding group in same columns.

The former is certainly not less variable than the latter, having regard to the probable error. The ratio of the difference in variability to its probable error is in no case greater than 2.5, a value too small to attach any significance to.

2. While in the last three series the sign of the difference is positive, indicating a greater variability of the CO_2 determination as compared with the O_2, the differences are so small in magnitude as not to differ significantly from zero.

Turning to the second question, the pertinent correlation coefficients are given in Table 2. In this table r denotes a coefficient of correlation between the two variables indicated by the attached subscript letters.

TABLE 2

	Direct calorimetry (10 cases)	Direct calorimetry (17 cases)
r_{AC}	+ .719± .103	+ .846± .047
r_{BC}	+ .488± .162	+ .836± .049

The significant comparisons are:

Direct calorimetry (10 cases)

$r_{AC} - r_{BC} = .719 - .488 = + .231 \pm .192$ Diff/P.E.$_{Diff} = 1.2$.

Direct calorimetry (17 cases)

$r_{AC} - r_{BC} = .846 - .836 = - .010 \pm .068$ Diff/P.E.$_{Diff} = 0.1$.

From these results it must be concluded that the O_2 intake is not significantly more highly correlated with directly measured calorie output than is CO_2. The differences in the correlations are sensibly zero, having regard for these probable errors. In the longer series (17 cases) the correlation is actually slightly (but not significantly) higher for the CO_2 with calories than for the O_2 with calories.

Method for Measuring Carbon-Dioxide

With the foregoing considerations in mind, it seemed reasonable to construct an apparatus for collecting and measuring carbon-dioxide elimination, with a view toward its possible use as a clinical index to the metabolic rate.

Such an apparatus is shown in Fig. 1, and is quite simple. The method is "open," the older U. S. Army gas-mask being used to deliver outside air to the subject and to carry expired air to the tubing leading to the three jars. The gas-mask canister, seen hanging from the horizontal bar, was emptied by removing the valve in the bottom, removing the contents and sealing the valve into place again. On the intake side, then, there is nothing between the patient and the outside air except a light rubber valve in the bottom of the canister, which rises and falls with respiration.[9]

Expired air passes through the "flutter" valve of the gas-mask and is caught in a rubber cuff, that is sealed with paraffin about this outlet valve. The cuff tapers into rubber tubing and the expired air passes through the three collecting jars.

Resistance to the passage of air through the jars has been measured by inserting a water manometer between the subject and the first jar. Observations upon two large men showed an average momentary rise of pressure of 6 to 8 mm. H_2O. On deep respiration the pressure rose occasionally with a quick elevation, such as one sees in a "water hammer" pulse, to 14 mm. H_2O. Such pressure changes, it was thought, made a fan superfluous. There is no inspiratory effort whatever.

The three jars are identical in construction and are such as were part of the original Benedict "portable" apparatus.

[9] The mouth-piece of the gas-mask is too small for this work and the larger mouth-piece made by the Sanborn Company, Boston, Mass., is used. It is removed and boiled for each test.

FIG. 1.—Apparatus and Balance for Measuring Carbon-Dioxide Elimination

Incoming air is carried through a tube almost to the bottom of the jar. Here it is scattered through a cone with multiple perforations, filters upward through the chemicals, and passes out at the top.

The first jar contains 4-mesh calcium chloride.[10] This has been found to desiccate expired air completely.

The second jar contains soda lime and absorbs carbon-dioxide from the dried air. Observations have been made upon the efficacy of this soda lime in repeated tests by attaching a soda lime and calcium chloride jar at the end of the usual series of three jars. It was found that the soda lime jar, after it has absorbed as much as 111 grms. CO_2 still completely removed CO_2 from the air. The writer breathed through the jars for 15 minutes after the soda lime had absorbed 111 grms. CO_2. He had not been prepared, was not quiet and his CO_2 output was 39 per cent above the average normal. No CO_2 was collected in the additional jars. CO_2 did, however, pass through the absorbing jars after 150 grms. CO_2 had been absorbed. To be perfectly safe we have become accustomed to change soda lime after it has taken up a total of 75 grms.[11]

The third jar contains calcium chloride of the same type as that used in the first jar. This third jar is necessary because the soda lime (jar II) contains a rather high percentage of moisture, some of which is carried out of jar II during each test; jar III absorbs this vapor. This increase in weight in jar III, therefore, must be added to the net increase in weight of jar II in order that the determination of CO_2 may be correct.

Technic of Test

The patient is prepared in the usual way, by the omission of breakfast and by half an hour's rest before the test. The room is well ventilated before and during the examination. The patient is carefully protected from cold.

The mouth-piece and nose-clip[12] are adjusted. The patient is instructed to be quiet and to breathe naturally. For several minutes a preliminary period is carried out, the patient breathing through the first jar only. This is done in order to allow the patient to become accustomed to the procedure, and the length of this preliminary period depends upon the readiness with which he settles down to the task. It is found that most complaints arise during the very beginning of the test. If a new start must be made, it is, therefore, usually accomplished during this preliminary period, and it is not necessary to reweigh jars II and III. As soon as the patient is quiet and breathing smoothly, jars II and III, which have been previously attached to each other, are joined to jar I, note being taken of the time—to the second. The expired air is passed through all these jars for ten minutes. Ten-minute periods are repeated until two of the results agree reasonably closely. The sum of the increase in weight in jars II and III represents the CO_2 elimination. The CO_2 for two periods is added and multiplied by 3. This gives CO_2 elimination per hour. This is divided by the body surface (DuBois Height-Weight Chart)[13]. The resultant figure—grms. CO_2 per sq. meter per hour—is our end result. This is compared with the standard normal figures and the percentage deviation from the average normal figure is calculated.

TABLE IV

THE CARBON-DIOXIDE ELIMINATION OF HEALTHY MEN AT REST (POST-ABSORPTIVE PERIOD)

THE AUTHOR'S NORMAL CONTROL OBSERVATIONS

3rd and 4th Decades

Name	Age	Height in cm.	Weight in kgm.	Body surface (Dubois formula)	A: CO_2 eliminated per hr. in gms.	A: CO_2 per hr. per sq. m. body surface (gms.)	Deviation of CO_2 from average (gms.)	Percent deviation of CO_2 from average
N. C.	33	180	75.9	1.95	23.88	12.25	—0.58	— 4.7
W. R.	25	180	73.2	1.925	24.08	12.51	—0.32	— 2.5
C. P.	28	177	71.4	1.87	23.25	12.43	—0.40	— 3.1
W. L.	33	178	72.7	1.90	23.40	12.32	—0.51	— 3.9
L. G.	27	179	61.4	1.77	21.36	12.07	—0.76	— 5.9
S. S.	25	172	66.4	1.78	22.76	12.79	—0.04	— 0.3
L. M.	26	190	81.8	2.09	27.96	13.38	+0.55	+ 4.3
V. M.	30	178	72.3	1.89	22.44	11.87	—0.96	— 8.1
J. K.	22	170	51.82	1.58	22.08	13.98	+1.15	+ 8.9
D. A.	29	175	60.5	1.74	25.64	14.74	+1.91	+14.9
G. G.	22	188	84.09	2.11	31.04	14.71	+1.88	+14.7
H. R.	29	177	65.9	1.81	23.40	12.93	+0.10	+ 0.8
H. J.	27	182	70.5	1.90	22.64	11.92	—0.91	— 7.1
G. H.	29	173	65.4	1.78	21.24	11.93	—0.90	— 7.0
P. C.	27	173	57.7	1.69	22.12	13.09	+0.26	+ 2.0
W. T.	27	168	62.5	1.72	20.80	12.09	—0.74	— 5.8
E. B.	30	185.5	76.3	1.99	26.24	13.19	+0.36	+ 2.8
Total....	469					218.20		
Average .	27.6					12.83		

Application of the Method

In order to test the method which has just been described a series of 17 observations was made upon normal subjects. Each observation included a preliminary period and 15 minutes of CO_2 collection. The results are shown in Table IV. The table shows that an average of 12.83 grms. CO_2 per square meter of body surface per hour is eliminated by healthy men whose ages lie between 20 and 40 years.

On the basis of this standard figure, a prediction was made by the writer of CO_2 elimination for various ages and for both sexes. This is tabulated in Table IV. These results are based upon the curve constructed by Aub and DuBois,[14] showing the heat production of healthy males at various ages. The figures for women were obtained by deducting 7 per cent from the figures for men. These predicted values are shown in Table V, side by side with the actual values observed by Benedict and his associates—obtained from the analysis of Benedict's figures by decades (Table III). The correlation between the two sets of figures will be seen to be close for the decades in which actual observations were made: those of

[10] $CaCl_2$ 4 mesh, purified, anhydrous, for drying purposes, purchased from J. T. Baker Chem. Co., Phillipsburg, N. J., in 5-pound jars.

[11] The soda lime used is the Wilson [navy] soda lime, purchased from the Dewey and Almy Chemical Co., N. Cambridge, Mass., in 45-pound cans. Professor Wilson, of the Massachusetts Institute of Technology, writes me that this soda lime is by far the most active preparation that is available for CO_2 absorbing purposes.

[12] The gas-mask nose-clip is excellent.

[13] Lusk: Science of Nutrition. W. B. Saunders Co., Philadelphia and London, 1919.

[14] *Loc. cit.*

Benedict and others are 13.13 grms. and 12.89 grms. for the third and fourth decades, respectively. The author's figure for the third and fourth decades together is 12.83 grms. A reasonably close correlation exists throughout the figures for women, again especially in the lower decades. The figures of the upper decades of man show discrepancies which may be explained by pointing out the relatively small number of observations by Benedict and others that fell within the upper decades.

TABLE V

Normal Standards of Carbon-Dioxide Elimination

Figures Represent Gms. CO_2 per Sq. Meter Body Surface (DuBois Formula) per Hour *

Age	Men			Women		
	Benedict, Emmes, Roth and Smith	The author	Average	Benedict, Emmes, Roth and Smith	The author	Average
15–20	14.16	13.90	14.03	12.57	12.93	12.75
20–30	13.13	12.83	12.98	11.89	12.02	11.95
30–40	12.89	12.83	12.86	11.85	11.86	11.85
40–50	11.88	12.52	12.20	11.43	11.74	11.58
50–60	11.38	12.21	11.79	11.76	11.37	11.56
60–70	11.19	11.86	11.52	.....	11.05	11.05
70–80	.....	11.53	11.53	9.47	10.71	10.14

Proposed Scale of Normal Figures

Age	Men	Women
15–20	14.03	12.75
20–30	12.98	11.95
30–40	12.86	11.85
40–50	12.52	11.74
50–60	12.21	11.37
60–70	11.86	11.05
70–80	11.53	10.71

* The author's standard figures were calculated from the findings on the 17 normal controls between 20 and 40 years. From these figures the expected CO_2 elimination for men and women at various ages was computed from the chart by Aub and DuBois,† showing metabolic rates at various ages.

† The Basal Metabolism of Old Men. Aub and DuBois. Arch. Int. Med., 1917, V. 19, Part ii.

A satisfactory scale of normal figures may therefore be obtained by taking an average between Benedict's and the author's figures for the lower decades. For decades above the fourth it seems to me safer to follow the CO_2 figures predicated upon the curve of Aub and DuBois. Such a proposed scale is shown at the bottom of Table V. It must be remembered, however, that considerable deviation from such figures must be expected, even in normal subjects, as shown by the Benedict reports of actual observations.

Discussion

Effect of Superventilation of the Lungs

It is generally believed by those interested in the study of gas exchange that CO_2 is eliminated in a manner less even than the absorption of O_2. We have considered this matter carefully and have obtained the following data.

It has been shown from the analysis of the 27 experiments with subjects in the chamber calorimeter that the co-efficient of correlation of CO_2 with calories is actually higher than that of O_2 with calories. So far, then, as this points the way, we may say that CO_2 furnishes a better index to the heat production (basal metabolism) than does O_2—that is, under ideal conditions. It also seems fair to say that, if any handicap in favor of CO_2 as an index to basal metabolism is found to exist under ideal conditions, then such a handicap must still be considered to play some rôle even under less favorable conditions.

Lusk, in the introduction of his *Science of Nutrition,*[15] quotes experiments of Lossen upon the CO_2 elimination during extreme variations of ventilation of the lungs. The subject voluntarily breathed at various rates between 75 and 182 liters of air per 15 minutes. The results showed that CO_2 elimination for 15-minute periods could not be altered by the rate of ventilation of the lungs except in so far as it might be raised by excessive activity of the respiratory muscles.

Let us consider the theoretical aspect. It is generally believed that respiration is caused by the circulation of CO_2 through the respiratory center, and that the absorption of O_2 is a purely passive act. Inasmuch as the act of gas exchange is caused by the CO_2 that results from oxidation and not by a demand for O_2, might we not assume that CO_2 elimination should be closely related in a quantitative way to heat production?

It remains, however, to investigate the CO_2 elimination during tests by the "indirect" method. The question is whether patients breathing through their mouths, with their nostrils closed, breathe so unnaturally as to "wash out" CO_2 that has previously been formed in the body, and so give a false index to the actual CO_2 formation and hence to the heat production. This can best be answered by actual tests. If CO_2 can be "washed out" by a nervous subject and O_2 not absorbed in a proportionate amount, then a handicap can be shown in favor of O_2 as an index. To determine this, let us examine the results of the tests carried out on 157 normal subjects by Benedict and his associates and shown in Table III. If "washing out" of CO_2 is a matter of practical importance, it would manifest itself by the CO_2 figures being much higher than the O_2 figures in corresponding experiments.

The average CO_2 elimination has been calculated according to decades and the average O_2 absorption similarly worked out. Next, the deviation of the CO_2 output from its average and the O_2 intake from its average were calculated for each subject.

Table VI shows the deviation of CO_2 per cent from the average in each case in which it was higher than the average. O_2 deviations are tabulated in a parallel column.

Table VI also shows that in all observations upon male subjects there is no tendency of the CO_2 output to be erratically higher than a corresponding O_2 consumption figure. Among the observations upon women one CO_2 figure is about 4 per cent higher than a corresponding O_2 figure and another one is about 2 per cent higher than a corresponding O_2 figure.

[15] *Loc. cit.*

TABLE VI

Per cent	Men		Women	
	CO_2	O_2	CO_2	O_2
Plus 22.....	....	....	1	....
Plus 20.....	....	....	....	....
Plus 18.....	....	....	1	1
Plus 16.....	....	....	....	1
Plus 14.....	1	1	....	....
Plus 12.....	111	111	11	1
Plus 10.....	1111	11	11	....
Plus 8.....	11111 1	111	1111	11111
Plus 6.....	11111	11111	1111	11111 1
Plus 4.....	11111 11111 11	11111 11111 11	1111	1111
Plus 2.....	111	11111 11111	11111	11111
Plus 0.....	11111	11111 111	11111 111	11111 1

The above table shows all positive deviations of CO_2 and of O_2 in 157 protocols (from Table III).

Out of 157 observations upon all types of normal subjects except the very young, only two showed CO_2 figures that ran distinctly ahead of corresponding O_2 figures, and in these two the deviation per cent was slight. Moreover, it is impossible to say, even in these cases, whether the O_2 or the CO_2 figure might be a better index to the heat production.

Another method of approach was used to investigate the possibility of "washing out." Observations were made upon ten unselected cases to test the effect of the preliminary period. In each case the first reading consisted of a 15-minute period, preceded by a 4-minute preliminary period; the second reading consisted of a 10-minute period without any preliminary period whatever. The results, shown in Table VII, demonstrate a negligible difference between the averages of the two sets of figures. The first two periods were used in each case, no attempt being made to select results from repeated observations.

TABLE VII

Showing the CO_2 Elimination Following a Preliminary Period (Col. 1) and Without Preliminary Period (Col. 2)

Grams CO_2 per hour (Total)

	Col. 1	Col. 2
	22.36	21.48
	18.88	18.78
	16.92	19.74
	29.32	27.66
	20.56	20.82
	25.92	24.48
	17.68	17.28
	19.44	22.20
	31.16	32.52
	24.76	23.52
Total	227.00	228.48
Average ..	22.70	22.85

Summary

To sum up, then, what are the advantages or disadvantages of using CO_2 elimination instead of O_2 consumption as an index to basal metabolism?

1. The apparatus needed to collect and weigh CO_2 is simple and stable.

2. The method is "open." This prevents danger of possible respiratory infection, for which the "closed" methods have been criticized.

3. By weighing the CO_2 output one needs to make no corrections for temperature and barometric pressure, such as becomes necessary in using volumetric methods of O_2 consumption.

4. The psychic effect upon the patient that accrues from this open method are as follows:

A. He may be assured that he is breathing "fresh air."

B. The moving spirometer and the buzzing fan, often part of the "closed" apparatus, may form an annoyance to the patient.

5. Statistical studies upon protocols of two groups of experiments with the Atwater chamber calorimeter show a somewhat higher coefficient of correlation between CO_2 and measured calories (plus .719 and plus .846 in the two series) than exists between O_2 and measured calories (plus .488 and plus .836 in the two groups.)

6. An analysis of 157 published observations upon gas exchange showed that CO_2 is either not "washed out" during the practical application of the basal metabolism test, or else it is "washed out" in negligible amounts.

7. Results of measurements of CO_2 obtained through the method suggested in the paper corresponded closely with those published by Benedict and associates.

8. The practical application of the proposed method has been satisfactory in several hundred observations upon all types of patients. The method should not be used in diabetes because of the altered respiratory quotient in that disease.

Conclusion

1. A method is proposed whereby CO_2 elimination may be used as an index to basal metabolism. The apparatus is stable, simple and relatively inexpensive.

2. The CO_2 elimination seems to be at least as accurate and possibly a more accurate index to heat production than is O_2 consumption.

I feel myself much indebted to Dr. W. W. Palmer for his helpful interest in this work.

THE LOCALIZATION OF BACTERIA IN THE UPPER AIR PASSAGES: ITS BEARING ON INFECTION

By Arthur L. Bloomfield

(From the Biological Division of the Medical Clinic, The Johns Hopkins University and Hospital)

Despite the large amount of clinical and experimental information which we possess about the mechanism of infection, most of the details of the problem remain unsolved. We know that organisms are transmitted from one person to another by direct or by indirect means, and that in some cases disease follows the acquisition of such organisms, but the exact conditions which underlie the production of disease in the individual are still obscure. In dealing with the process of an infection entering the body through the respiratory tract we have to reckon with several distinct stages: first, the organism must be transported from some outside source to the individual to be infected; secondly, the organism after reaching the upper air passages must colonize there and not be immediately disposed of; and thirdly, it must invade the body, thus leading to the production of disease. Obviously the last two stages may, at times, be simultaneous.

It seems to us that little or no further advance in the solution of the problem of respiratory infection can be made unless we acquire accurate detailed information about the mechanism of these various stages. The laws governing the disposal of bacteria in the upper air passages, the factors which make possible colonization, and the influences which regulate the duration of carrier states must be worked out. During the past two years we have attempted to collect information of this sort by working along several lines. First, a study was made of the fate of various organisms experimentally introduced into the upper air passages.[1] The general result of this work was to the effect that foreign organisms, as a rule, are promptly eliminated, and that they can no longer be recovered in cultures made from the site of inoculation after 48 hours. It was also possible to show that the mechanism responsible for this disposal was the flushing action of the mouth secretions. The correctness of this principle has been checked in two ways: first, by clinical observation which shows that acquisition of respiratory disease often fails to occur even on prolonged and close contact exposure (for example, in lobar pneumonia); and secondly, by serial cultural studies of the throat floras of people exposed to respiratory disease which remain remarkably constant in spite of such exposure. In addition to the above-mentioned work a detailed study of the throat floras of individuals clinically normal was made.[2] Serial cultures revealed three distinct groups of organisms: first, a constant basic flora continuously present over long periods of time consisting of non-hemolytic streptococci, Gram-negative cocci, and probably diphtheroids; secondly, a transient flora of non-pathogenic or potentially pathogenic bacteria present only for short periods of time and corresponding in their behavior to that of organisms experimentally introduced; and thirdly, foreign organisms which were "carried" over long periods of time.

It seemed that the next step in the problem was to determine more accurately the mode of growth in the upper air passages of these various groups of bacteria, to discover whether they grow at large over the free surfaces of the mucous membranes or whether they are confined to special localities. This point is of especial importance in connection with carriers of pathogenic organisms. If it could be shown, as has been suggested by other observations,[3] that a chronic carrier state implies the presence of a local focus of diseased tissue in which the bacteria are breeding, the ground will be cleared for a study of the factors underlying the production of such foci.

The present study concerns itself, therefore, with the results of serial differential cultures from various parts of the upper air passages.

Material

Eight clinically healthy persons were studied. They were all workers in the bacteriological laboratory and were exposed to infectious diseases of various sorts in the wards.

Methods

Five swabs were taken simultaneously from the tongue, posterior pharyngeal wall, each tonsil, and the anterior nares. In the last case an effort was made to avoid touching the nasal orifice, and the swab was introduced about 3 cm. The swabs were spread on rabbit blood agar plates by the method already described[2] and the cultures were studied qualitatively and quantitatively.

Results

The results will be discussed from two points of view—that of various areas in the upper air passages, and that of individual organisms.

1. *The Nose.*—Staphylococcus albus was uniformly present in every culture, varying in number from a few to several hundred colonies. It seems clear that this organism may be regarded as a constant normal finding in this situation. Whether it actually lives and grows on the nasal mucosa or whether it is simply introduced from the adjacent skin surfaces one cannot say definitely. The fact that the deeper nasal passages show fewer organisms inclines us to the latter view. The white staphylococci found were both hemolytic and non-hemolytic. Diphtheroid bacilli were found in about three-fourths of the cultures. Several types were met with, and again it seems likely that they are swept in from adjacent skin surfaces. In addition to the above organisms, several other

varieties were recovered which were definitely present as transients accidentally introduced. Thus Case VIII showed five colonies of B. lactis aërogenes on one culture; Case V, six colonies of Staph. aureus on one culture; Case III, Staph. aureus on two cultures (one and two colonies, respectively); Case II, Gram-negative cocci (2 cols.) in one culture, a Gram-positive bacillus (15 cols.) in one culture; and Case VII showed Beta hemolytic streptococci on two occasions evidently introduced from the mouth, and Staph. aureus (3 cols.) at another time. Case IV carried a Staph. aureus constantly without obvious disease.

In summary, then, Staph. albus was constantly found in the nose, diphtheroids of various sorts almost constantly, and a variable transient flora of pathogenic and non-pathogenic bacteria.

2. *The Tongue.*—Gram-negative cocci of various sorts are the predominating bacteria in all the cultures. They are constantly present and clearly are members of the normal flora. Non-hemolytic streptococci are also constantly present but in somewhat smaller numbers. Diphtheroids of various sorts were encountered in about half the cultures. In no cases was any other organism repeatedly recovered from the tongue, though a few transients were picked up on single occasions—hemolytic streptococcus (once), a large Gram-positive coccus (once), Staph. albus (twice) and hemolytic influenza bacilli (eight times).

In summary, then, the flora of the tongue save for an occasional transient is simple and constant. This is to be expected, inasmuch as the anatomical structure is not such as would promote the possibility of carrier states as is so in the case of the tonsil.

3. *The Tonsils.*—Cultures from the tonsils were of special interest, since it became clear that when foreign organisms were carried their breeding place was usually here. Thus, Case V carried hemolytic streptococcus; Case I, hemolytic streptococcus; and Case II, Staph. aureus. Gram-negative cocci, non-hemolytic streptococci and diphtheroids (the normal flora) were constantly present unless obviously displaced by some foreign organism. A transient group of organisms was also encountered—Staph. albus, Staph. aureus, hemolytic streptococci, hemolytic influenza bacilli, a Gram-negative branching organism, etc.—usually in small numbers and in single cultures. It is of interest that the type of Staph. albus recovered (hemolytic or non-hemolytic) usually corresponded with that found in the nose, suggesting that the few found in the pharynx are swept back from the nose.

In summary, then, the tonsils show normal flora, transients and organisms which are carried in diseased tissue.

4. *The Pharynx.*—Conditions here correspond to those found in the case of the tonsils.

From the standpoint of individual organisms the following points may be brought out:

1. *The Gram-Negative Cocci.*—The present series of differential cultures supports the idea gained from previous work[3] that this group of organisms are normal basic inhabitants of the mouth and throat. Their wide distribution in these areas and their constant presence lead us to believe that they may lead an unaided parasitic existence on the normal mucous surfaces. This does not imply that they may never play a part in disease processes, but it seems likely that they act as secondary invaders and not as primary incitants of disease. The meningococci are for the time being excluded from these considerations.

2. *The Non-Hemolytic Streptococci.*—This group of organisms seems also definitely to fall into the normal constant flora. As seen from the tables, they are found uniformly spread through the mouth cavity, as indicated by cultures from tongue, tonsils and pharynx. We wish especially to call attention to frequent instances in which practically pure plates of these organisms are recovered from healthy individuals. It is not our purpose at present to discuss the relation of non-hemolytic streptococci to disease—the point of importance is that their mere presence in itself does not indicate an etiological relationship. A wide variety of strains is encountered judging by colony formation and morphology.

3. *Diphtheroid Organisms.*—This group was encountered in the majority of the cultures. We are inclined, despite the fact that they are not invariably present, to class them as normal inhabitants. They are for the most part slow-growing organisms, and special methods to prevent overgrowth by other bacteria show them to be almost constantly present. No attempt was made to work out the varieties of this group in detail—many kinds of diphtheroids are present in the upper air passages. Three types are especially frequent, a small, flat, dry, grey colony consisting of long forms, a glistening, grey-white bead-like colony of short stubby forms, and a very small, tough, stellate, brownish colony of minute organisms. No suggestion of pathological activity was gained from our cultures. Their significance in the upper air passages is probably similar to that elsewhere in the body.[4]

4. *Staph. albus.*—This organism, as was brought out above, is a normal habitant of the nose. It is not, however, normally present with constancy in the mouth. A few colonies were encountered from time to time clearly in the nature of transients introduced from the skin or nose. This organism may occasionally be associated with local infections (in the sinus, tonsil, etc.), in which case it is persistently present and usually in large numbers.

5. *Staph. aureus.*—This organism is not a normal inhabitant of the upper air passages. We have encountered it under two conditions: first, as a transient in nose or throat (Cases III and V); and secondly, associated with a chronic focal infection (Case II).

6. *Influenza Bacilli.*—Their significance and that of the pneumococci will be discussed in another paper.

7. *Beta Hemolytic Streptococci.*—These we have found (1) associated with acute tonsillitis or acute infections, such as influenza and scarlet fever; (2) in chronically diseased tonsils; and (3) occasionally as transients (Case VII).

The detailed results of the cultures follow:

CASE I—F

Culture	March 12	March 14	March 16	March 18
Nose	Staph. albus †(4)	Staph. albus (4)	Staph. albus (5) Beta hemol. strept. *(∞)	Staph. albus (2) Beta hemol. strept. (3) Spreader (1)
Pharynx	Beta hemol. strept. (x) Gram-neg. cocci (6) Streptococcus-grey (6) Diphtheroid (a few)	Beta hemol. strept. (∞) Gram- neg. cocci (a few) Strept.-grey (many)	Beta hemol. strept. (x) Strept. non-hemol. (a few) Staph. albus (1)	Beta hemol. strept. (∞) Gram-neg. cocci (a few) Diphtheroids (a few) Staph. albus (2)
Right tonsil	Beta hemol. strept. (200) Gram neg. cocci (x) Streptococcus-grey (many) Staph. albus (1) Staph. aureus (1) Diphtheroids (1)	Beta hemol. strept. (∞) Gram-neg. cocci (many) Strept.-grey (many)	Beta hemol. strept. (∞) Gram-neg. cocci (many) Strept. non-hemol. (many) Staph. aureus (1) Diphtheroids (many)	Beta hemol. strept. (∞) Gram-neg. cocci (many) Strept. non-hemol. (many) Staph. albus (1)
Left tonsil	Beta hemol. strept. (100) Gram-neg. cocci (sev. hundred) Streptococcus-grey (x)	Beta hemol. strept. (∞) Gram-neg. cocci (x) Strept.-grey (many) Strept.-green (a few) Diphtheroids (a few)	Beta hemol. strept. (∞) Gram-neg. cocci (a few) Strept.-grey (a few) Strept.-green (a few) Diphtheroids (a few)	Beta hemol. strept. (many) Gram-neg. cocci (x) Strept.-grey (many) Strept.-green (a few)
Tongue	Gram-neg. cocci (x) Streptococcus-grey (many) Streptococcus-green (a few) Diphtheroids (many) Hemol. influ. bac. (a few)	Gram-neg. cocci (∞) Strept.-grey (many) Strept.-green (a few) Hemol. influ. bac. (2)	Gram-neg. cocci (∞) Strept.-grey (many) Strept.-green (many)	Gram-neg. cocci (∞) Strept.-grey (many) Strept.-green (many)
Remarks	Just over a mild tonsillitis. Throat looks clear, but complains of rawness. Small clean tonsils.	Cold in nose.	Cold improving.	Well.
Culture	**March 29**	**April 12**	**May 13**	
Nose	Staph. albus (50)	Staph. albus (12) Staph. aureus (3)	Staph. albus (8) Diphtheroids (many)	
Pharynx	Beta hemol. strept. (4) Strept. non-hemol. (many) Staph. albus (6) Gram-neg. spore bear. bac. (6)	Beta hemol. strept. (∞) Gram-neg. cocci (1) Strept. non-hemol. (a few) Staph. albus (1)	Beta hemol. strept. (50) Gram-neg. cocci (a few) Strept. non-hemol. (x) Diphtheroids (a few) Hemol. influ. bac. (many)	
Right tonsil	Beta hemol. strept. (many) Gram-neg. cocci (a few) Strept. non-hemol. (many) Diphtheroids (a few)	Beta hemol. strept. (many) Gram-neg. cocci (many) Strept. non-hemol. (many)	Beta hemol. strept. (sev. hundred) Gram-neg. cocci (many) Strept. non-hemol. (many) Diphtheroids (a few)	
Left tonsil	Beta hemol. strept. (sev. hundred) Gram-neg. cocci (many) Strept.-grey (many) Strept.-green (sev. hundred)	Beta hemol. strept. (very many) Gram-neg. cocci (a few) Strept.-grey (x) Strept.-green (x) Diphtheroids (a few)	Beta hemol. strept. (sev. hundred) Gram-neg. cocci (a few) Strept.-grey (many) Strept.-green (many) Diphtheroids (a few)	
Tongue	Gram-neg. cocci (x) Strept.-grey (a few) Strept.-green (very few)	Gram-neg. cocci (∞) Strept.-grey (many) Strept.-green (a few) Diphtheroids (a few) Hemol. influ. bac. (20)	Gram-neg. cocci (x) Strept.-grey (a few) Strept.-green (a few) Hemol. influ. bac. (40)	
Remarks	Well.	Well.	Well.	

* x = innumerable.

† The number of colonies is indicated in brackets.

Discussion of Case I.—This individual—a member of the hospital staff—showed no outspoken abnormalities. The nose and throat were clinically normal, save for moderately enlarged scarred tonsils. At the beginning of our period of observation he was convalescent from a mild attack of tonsillitis. A culture made one week previously had shown no hemolytic streptococci but innumerable colonies of hemolytic influenza bacilli. The cultures bring out the following points: (1) Staph. albus was constantly present in the nose; streptococcus, aureus, spreaders, and diphtheroids were met as transients. Staph. albus was at times present in small numbers in the pharynx—evidently washed back from the nose. Gram-negative cocci were almost constantly present in the pharynx, tonsils, and tongue. The same was true of the non-hemolytic streptococci. Diphtheroids were found at one or another site on each culture. Staph. aureus was twice found in small numbers in the pharynx—evidently a transient. Hemolytic influenza bacilli were found on the tongue four times, in the pharynx once. Beta hemolytic streptococci were present in large numbers in the pharynx and on both tonsils in every culture during the period of observation covering two months. It seems clear that this organism was the cause of the patient's tonsillitis and that a carrier state followed. It is of interest that the organism remained confined to a limited region and was not disseminated throughout the mouth cavity, cultures from the tongue being always negative. The presence of the hemolytic streptococcus in the nose for a few days is of interest. This was clearly an auto-inoculation, and the organism was present simply as a transient without colonization. The same has been noted in the case of other carriers.

In summary, then, the case shows the normal flora plus a Beta hemolytic streptococcus which has persisted in the tonsils following tonsillitis.

CASE II—B

Culture	March 19	March 23	March 28	April 11	May 15
Nose	Staph. albus (100)	Staph. albus (60) Diphtheroids (20) Gram-neg. cocci (2)	Staph. albus (sev. hundred) Diphtheroids (∞) Long thread-like (16) (Gram-pos. bacillus)	Staph. albus (150) Spreaders (a few)	Staph. albus. (∞) Diphtheroids (a few)
Pharynx	Gram-neg cocci (∞) Non-hemol. strept. (∞) Staph. albus (a few) Diphtheroids (a few)	Gram-neg. cocci (a few) Non-hemol. strept. (many) Staph. albus (a few) (Gram-neg.) (50) (spore-bearer)	Gram-neg. cocci (many) Non-hemol. strept. (many) Staph. albus (2) Diphtheroids (many) *Staph. aureus* (2)	Gram-neg. cocci (very many) Non-hemol. strept. (a few) Diphtheroids (a few) *Staph. aureus* (4)	Gram-neg. cocci (many) Non-hemol. strept. (many) Diphtheroids (a few) *Staph. aureus* (1)
Right tonsil	*Staph. aureus* (100) Gram-neg. cocci (many) Non-hemol. strept. (many)	*Staph. aureus* (∞) Gram-neg. cocci (a few) Non-hemol. strept. (a few) Diphtheroids (a few)	*Staph. aureus* (∞) Gram-neg. cocci (few) Non-hemol. strept. (many) Diphtheroids (a few)	*Staph. aureus* (50) Gram-neg. cocci (many) Non-hemol. strept. (many) (A few cols. of a Gram-neg. branching organism)	*Staph. aureus* (∞) Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few)
Left tonsil	*Staph. aureus* (200) Gram-neg. cocci (many) Non-hemol. strept. (many) Diphtheroids (a few)	*Staph. aureus* (∞) Gram-neg. cocci (a few) Non-hemol. strept. (a few)	*Staph. aureus* (very many) Gram-neg. cocci (a few) Non-hemol. strept. (very many) Diphtheroids (a few)	*Staph. aureus* (∞) Gram-neg. cocci (very many) Non-hemol. strept. (many)	*Staph. aureus* (4) Gram-neg. cocci (many) Non-hemol. strept. (many) Diphtheroids (a few)
Tongue	Gram-neg. cocci (∞) Non-hemol. strept. (a few) Hemol. influenza bac. (2)	Gram-neg. cocci (∞) Staph. albus (2)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Hemol. infl. bac. (50)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few)	Gram. neg. cocci (∞) Non-hemol. strept. (a few)
Remarks	Nose and throat negative. Small clean tonsils.	Onset of severe coryza (Mar. 22). Malaise, dullness, rawness of nose and throat. Nasal occlusion with thick mucoid discharge.	Well.	Well.	Well.

Discussion of Case II.—This person was a normal control. There was no abnormality of the upper air passages save small scarred tonsils of no apparent clinical significance. Four cultures taken from this patient three months previously during the course of a cold had yielded a normal flora plus many colonies of Staph. aureus. Differential cultures were not made at the time. At the time of culture I of the present series (March 19), the patient was perfectly well. It is to be noted that the Staph. aureus was still present in the tonsils in large numbers. On March 22 a severe cold developed. It was clinically uncomplicated and the flora remained unchanged; *i. e.*, a cold occurring in a carrier of Staph. aureus. Had a single culture been made say on March 23 the state of affairs could not possibly have been interpreted. The origin of this staphylococcus carrier state is obscure. It is of interest that despite the abundance of the organisms on the tonsils there was no spread to surrounding areas. This suggests that the staphylococci were breeding in the tonsil and being discharged from within and were kept relatively localized by the overlying layer of mucus. Organisms introduced from without are promptly spread over the whole mouth cavity.

Aside from the above points nothing remarkable is noted in this case. The normal constant flora, and a few insignificant transients were recovered.

CASE III—E

Culture	March 21	March 31	April 11	April 24
Nose	Staph. albus (6) Staph. aureus (2)	Staph. albus (6) Diphtheroids (∞)	Staph. albus (20) Staph. aureus (1)	Staph. albus (many) Diphtheroids (many)
Pharynx	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few)	Non-hemol. strept. (a few) Diphtheroids (a few) Staph. aureus (∞)	Gram-neg. cocci (a few) Non-hemol. strept. (many) Diphtheroids (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few)
Right tonsil	Gram-neg. cocci (∞) Non-hemol. strept. (∞)	Gram-neg. cocci (many) Non-hemol. strept. (many) Diphtheroids (a few) Staph. albus (10) Staph. aureus (2)	Gram-neg. cocci (very many) Non-hemol. strept (∞)	Gram-neg. cocci (a few) Non-hemol. strept. (many) Staph. albus (4) Staph. aureus (2)
Left tonsil	Gram-neg. cocci (a few) Non-hemol. strept. (∞) Staph. albus (2)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (many) Hemol. influenza bac. (a few) B. hemol. strept. (a few)	Gram-neg. cocci (very many) Non-hemol. strept. (many) Staph. albus (1) Diphtheroids (a few)	Gram-neg. cocci (many) Non-hemol. strept. (many)
Tongue	Gram-neg. cocci (∞) Non-hemol. strept. (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (a few) Staph. albus (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (a few)	Gram. neg. cocci (∞) Non-hemol. strept. (a few) Diphtheroids (a few) Hemol. influenza bac. (3)
Remarks	Small scarred tonsils, clean superficially. Well at present.	Well.	Well.	Well.

Discussion of Case III.—This man has been under observation for over a year. During February, 1921, he had a mild attack of influenza during which a hemolytic streptococcus, which had not been present previously, appeared and persisted for about a month. At the time of the present series of cultures the patient was clinically well. The normal flora and the usual number of transients were found. On March 31 an almost pure plate of Staph. aureus was recovered from the pharynx, but this organism was found at no time thereafter. No clinical manifestations accompanied its presence. The few colonies of Beta hemolytic streptococcus present in the left tonsil on this day probably indicate a persistent infection following the attack in February. This case illustrates well the importance of serial quantitative cultures in interpreting the results of throat cultures.

CASE IV—M

Culture	March 22		April 4		April 16		May 16	
Nose	Staph. albus Staph. aureus Spreaders	(a few) (∞) (a few)	Staph. albus Staph. aureus	(∞) (20)	Staph. albus Staph. aureus Diphtheroids	(20) (100) (a few)	Staph. albus Staph. aureus Diphtheroids	(many) (many) (a few)
Pharynx	Hemol. influenza bac. Non-hemol. strept. A large G+ coccus	(40) (many) (a few)	Hemol. influ. bac. Non-hemol. strept. Gram-neg. cocci Staph. albus	(a few) (many) (many) (1)	Hemol. influ. bac. Non-hemol. strept. Gram-neg. cocci	(∞) (many) (many)	Hemol. influ. bac. Non-hemol. strept. Gram-neg. cocci Staph. albus	(sev. hundred) (a few) (many) (1)
Right tonsil	Non-hemol. strept. Gram-neg. cocci	(∞) (a few)	Non-hemol. strept. Gram-neg. cocci Hemol. influ. bac.	(∞) (a few) (many)	Non-hemol. strept. Gram-neg. cocci Hemol. influ. bac.	(many) (many) (∞)	Non-hemol. strept. Gram-neg. cocci Hemol. influ. bac.	(few) (many) (∞)
Left tonsil	Non-hemol. strept. Gram-neg. cocci A large G+ coccus Staph. albus Hemol. influenza bac.	(∞) (a few) (a few) (a few) (1)	Non-hemol. strept. Gram-neg. cocci Hemol. influ. bac.	(∞) (many) (many)	Non-hemol. strept. Gram-neg. cocci Hemol. influ. bac.	(∞) (many) (very many)	Non-hemol. strept. Gram-neg. cocci Hemol. influ. bac.	(many) (many) (many)
Tongue	Gram-neg. cocci Non-hemol. strept.	(∞) (a few)	Gram-neg. cocci Non-hemol. strept. A large G+ coccus	(many) (a few) (∞)	Gram-neg. cocci Non-hemol. strept.	(∞) (many)	Gram-neg. cocci Non-hemol. strept.	(∞) (a few)
Remarks	Well.		Well.		Well.		Well.	

Discussion of Case IV.—This person—a glassware washer in the laboratory—had been under observation all winter. She had no clinical respiratory infection of any sort. No abnormality was made out in the nose or throat save small clean tonsils. The cultures showed the members of the normal flora. In addition hemolytic influenza bacilli were constantly present in the pharynx and tonsils. The patient also carried Staph. aureus constantly in the nose. This was unassociated with any obvious infection. Diphtheroids were unusually scanty. A few insignificant transients were encountered.

CASE V—H

Culture	April 8		April 12		April 19			
Nose	Staph. albus Diphtheroids	(4) (20)	Staph. albus Diphtheroids (Gram-neg.)	(3) (100)	Staph. albus Diphtheroids Staph. aureus	(100) (6) (6)	Staph. albus Diphtheroids	(∞) (a few)
Pharynx	B. hemol. strept. Gram-neg. cocci	(∞) (a few)	B. hemol. strept. Gram-neg. cocci Non-hemol. strept. Diphtheroids	(20) (∞) (many) (a few)	B. hemol. strept. Gram-neg. cocci Non-hemol. strept. Diphtheroids	(∞) (a few) (many) (many)	B. hemol. strept. Gram-neg. cocci Non-hemol. strept. Staph. albus	(a few) (many) (a few) (10)
Right tonsil	B. hemol. strept. Gram-neg. cocci Non-hemol. strept. Diphtheroids	(∞) (a few) (a few) (a few)	B. hemol. strept. Gram-neg. cocci Non-hemol. strept.	(∞) (many) (a few)	B. hemol. strept. Gram-neg. cocci Non-hemol. strept. Diphtheroids	(∞) (many) (many) (a few)	B. hemol. strept. Gram-neg. cocci Non-hemol. strept. Diphtheroids	(∞) (many) (a few) (many)
Left tonsil	B. hemol. strept. Gram-neg. cocci Non-hemol. strept. Diphtheroids	(∞) (a few) (a few) (a few)	B. hemol. strept. Gram-neg. cocci Non-hemol. strept. Diphtheroids	(very many) (very many) (a few) (a few)	B. hemol. strept. Gram-neg. cocci Non-hemol. strept. Diphtheroids	(∞) (∞) (many) (a few)	B. hemol. strept. Gram-neg. cocci Non-hemol. strept. Diphtheroids Staph. aureus	(∞) (many) (a few) (many) (2)
Tongue	Gram-neg. cocci Non-hemol. strept.	(∞) (a few)	Gram-neg. cocci Non-hemol. strept. Diphtheroids Hemol. influ. bac.	(∞) (a few) (a few) (100)	Gram-neg. cocci Non-hemol. strept. Diphtheroids	(∞) (a few) (a few)	Gram-neg. cocci Non-hemol. strept. Diphtheroids	(∞) (a few) (many)
Remarks	Scarlet fever. Acute stage.		Throat still red.		Well.		Well.	

Discussion of Case V.—This patient developed a sore throat on April 5. On April 7 a typical scarlet fever rash appeared. She did not feel very ill, and remained at work. On April 8 the rash was well out, the throat looked diffusely red, the tonsils were large, red and ragged. There was no fever at this time. The point of interest in the cultures was the constant presence of Beta hemolytic streptococci on the pharynx and both tonsils, persisting during the whole period of observation. Aside from this the normal flora and the usual number of transients were found. Hemolytic influenza bacilli were recovered from the tongue in one culture.

CASE VI—D

Culture	April 8	April 18	May 13	May 17
Nose	Staph. albus (40) Diphtheroids (many)	Staph. albus (1) Spreaders (1)	Staph. albus (75) Diphtheroids (∞)	Staph. albus (75) Diphtheroids (a few)
Pharynx	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few)	Gram-neg. cocci (a few) Non-hemol. strept. (a few) Spreaders (1)	Gram-neg. cocci (a few) Non-hemol. strept. (a few) Diphtheroids (a few) Staph. albus (10)	Gram-neg. cocci (many) Non-hemol. strept. (many) Staph. albus (1)
Right tonsil	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few)	Gram-neg. cocci (many) Non-hemol. strept. (many) Diphtheroids (a few) Hemol. influenza bac. (a few) Alpha hemol. strept. (50)	Gram-neg. cocci (many) Non-hemol. strept. (many) Diphtheroids (many)
Left tonsil	Gram-pos. coccus (∞) B. influenzæ (∞)	Gram-neg. cocci (∞) Non-hemol. strept. (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few) Alpha hemol. strept. (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (a few) Diphtheroids (a few)
Tongue	Gram-neg. cocci (∞) Non-hemol. strept. (a few) Diphtheroids (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (a few) Diphtheroids (a few)

Discussion of Case VI.—This patient has been under observation for some time. He is subject to frequent respiratory infections and has chronically diseased tonsils with large infected crypts. The cultures show nothing remarkable save a rather abundant transient flora in addition to the normal organisms.

CASE VII —B1

Culture	April 13	April 20	April 29	May 6	May 17
Nose	Staph. albus (35) Diphtheroids (many)	Staph. albus (120) Diphtheroids (6)	Staph. albus (15) Diphtheroids (a few)	Staph. albus (300) Diphtheroids (∞)	Staph. albus (200) Diphtheroids (a few)
Pharynx	Gram-neg. cocci (∞) Non-hemol. strept. (many) Staph. albus (1)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (many)	Gram-neg. cocci (∞) Non-hemol. strept. (∞) Staph. aureus (1)	Gram-neg. cocci (many) Non-hemol. strept. (v. many) Diphtheroids (very many) Staph. aureus (200)	Non-hemol. strept. (a few) Staph. aureus (∞)
Right tonsil	Spreaders (?)	Gram-neg. cocci (∞) Non-hemol. strept. (∞) Staph. albus (1) Staph. aureus (2)	Gram-neg. cocci (∞) Non-hemol. strept. (∞) Diphtheroids (many)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Hemol. influ. bac. (4) Beta hemol. strept. (10) Diphtheroids (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (a few)
Left tonsil	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (∞) Diphtheroids (a few) Staph. albus (1)	Gram-neg. cocci (∞) Non-hemol. strept. (∞) Diphtheroids (many)	Gram-neg. cocci (∞) Non.hemol. strept. (many) Diphtheroids (a few) Staph. albus (1)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few) Staph. albus (3)
Tongue	Gram-neg. cocci (∞) Non-hemol. strept. (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few) Beta hemol. strept. (4)	Gram-neg. cocci (∞) Non-hemol. strept. (a few) Diphtheroids (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (a few) Diphtheroids (a few)

Discussion of Case VII.—This person is a laboratory worker who has been under observation over a year. He has small scarred tonsils, and has had no clinical respiratory infection during the past winter. The normal flora and the usual transients were present. In addition Beta hemolytic streptococci were recovered twice in small numbers, once from the tongue and once from the tonsil. It is of interest that this man was spending his entire time working with hemolytic streptococci. A few organisms were probably accidentally introduced into the mouth from time to time but never colonized. They fall into the group of transients. The appearance of a Staphylococcus aureus in the pharynx on three successive cultures over a period of three weeks without any clinical disease is also of interest. Had a respiratory infection developed at this time one would have been inclined to attribute it to this organism.

CASE VIII—M

Culture	April 14	April 20	April 27	May 6
Nose	Staph. albus (50) Diphtheroids (150) Lactis aërog. (5)	Staph. albus (25) Diphtheroids (a few)	Staph. albus (60)	Staph. albus (many) Diphtheroids (∞)
Pharynx	Strept. non-hemol. (∞) Hemol. influ. bac. (25)	Strept. non-hemol. (∞) Gram-neg. cocci (many)	Strept. non-hemol. (many) Gram-neg. cocci (a few) Staph. albus (1)	Strept. non-hemol. (∞) Gram-neg. cocci (∞)
Right tonsil (fossa)	Gram-neg. cocci (∞) Non-hemol. strept. (∞) Diphtheroids (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (∞)	Gram-neg. cocci (∞) Non-hemol. strept. (many)	Gram-neg. cocci (∞) Non-hemol. strept. (a few)
Left tonsil (fossa)	Gram-neg. cocci (∞) Non-hemol. strept. (∞) Diphtheroids (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (many)	Gram-neg. cocci (∞) Non-hemol. strept. (many)	Gram-neg. cocci (many) Non-hemol. strept. (many) Diphtheroids (a few) Staph. albus (1)
Tongue	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (a few)	Gram-neg. cocci (∞) Non-hemol. strept (a few)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (many)	Gram-neg. cocci (∞) Non-hemol. strept. (many) Diphtheroids (many)
Remarks	Well.	Well.	Well.	Well.

Discussion of Case VIII.—This individual had had the tonsils completely removed. The normal flora and a few transients were present.

Discussion

The present work adds to the information already collected bearing on the significance of the bacteria found in the upper air passages under various conditions. Topographical cultures confirm the previous observations in regard to the normal flora and bring out the important point that the constant normal inhabitants are widely and more or less uniformly distributed through the mouth and pharynx. Other organisms occur under different conditions which have been previously outlined.[5]

The general bearings of these studies on the question of the mechanism of respiratory infection seem to us to be as follows:

(1) Aside from the normal flora, bacteria do not, as a rule, grow free on the mucous surfaces of the upper air passages.

(2) Special conditions are necessary to account for the presence of foreign organisms—either a local infection, or a transient invasion.

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THE ICE-BOX MODIFICATION OF THE WASSERMANN TEST IN THE DIAGNOSIS AND TREATMENT OF SYPHILIS[1]

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The ice-box modification of the Wassermann test consists in conducting the preliminary incubation of serum, antigen, and complement for a period of several hours at temperatures approximating 8°C., or less. This modification has been employed because of its apparent greater sensitiveness in detecting fixing substances in the sera of syphilitics. The underlying factor or factors that create this greater sensitiveness are not as yet fully understood. Several views have been advanced. It has been suggested, for example, that there are variations in the velocity and degree of complement fixation at different temperatures; that prolongation of the incubation period, such as is necessary for the ice-box test, permits more complete fixation because of the greater time allowance; that incubation of complement in the presence of antigen produces greater deterioration of the complement in the cold than incubation at a temperature of 38°C. These and other hypotheses have been investigated by various workers, but although valuable data covering these points have been obtained, a complete solution has not as yet been reached.

The literature dealing with the experimental side of the problem has recently been reviewed by Kolmer[2], who also deals with the value of the ice-box modification in the diagnosis of syphilis. As we are not prepared at this time to express opinions based on experimental evidence concerning the nature of the test, we purposely avoid a discussion of the experimental literature. This paper deals with the ice-box test from the view-point of its practical application in the diagnosis, and more especially as a guide in the treatment of syphilis.

[1] Read before the annual meeting of the Medical and Chirurgical Faculty of Maryland, April 28, 1921.

[2] Kolmer, J. A., and Rule, A. M.: Studies in the Standardization of the Wassermann Reaction: XIV. The Influence of Temperature and Duration of Primary Incubation upon the Hemolytic Activity of Complement. Am. Jour Syph., 1920, IV, 675.

In order properly to orient ourselves, however, we considered it necessary to determine the status of several points of contention raised by various workers in this field, and in particular, whether false positive results are obtained by ice-box incubation; and whether the results obtained with a plain alcoholic antigen in the ice-box are equal to, or better than, those obtained with a cholesterinized antigen in the water-bath.

We have therefore examined by the ice-box method 300 cases in parallel series with Wassermann tests performed according to the original technique. Our purpose is to show to what extent the two tests are in agreement with each other and with the clinical diagnoses in this series of cases; and where this agreement was not obtained, we wish to analyse the clinical evidence in so far as it supports one test or the other.

The cases are divided into three groups on the basis of clinical diagnosis and previous water-bath Wassermann tests. It is assumed that with a properly controlled technique positive water-bath Wassermanns are to be relied on for a diagnosis of syphilis. The first group contains 107 individuals composed of 30 normal persons and 77 patients with clinical non-syphilitic diseases. In the second group are 176 syphilitic patients. The diagnosis of syphilis is based on clinical, serological, and anamnestic evidence. The third group contains 17 cases, including 16 in which the diagnosis of syphilis is considered but cannot be confirmed or ruled out on the basis of clinical and other serological evidence, and one apparently normal individual with an unconfirmed history of syphilis 21 years ago.

In performing the two series of tests identical "set ups" are employed and the series are done with the same preparations on the same days, except that on account of the longer incubation in the ice-box the second incubation for completing

these tests is performed the next day. Both plain and cholesterinized antigens are employed in the two series, the cholesterinized antigen being prepared from the identical plain extract with the addition of 0.2 per cent cholesterin. The anti-sheep hæmolytic system is used, and the technique of performing the tests has not been varied from that in general use in our laboratory during the past 7 years. Results are recorded as positive, suggestive positive, doubtful, suggestive negative, and negative. Complete fixation only is recorded as positive. Incomplete reactions are recorded in the descriptive terms mentioned. If numerals are substituted for these terms the scale runs from zero, indicating negative, to 4, indicating positive.

Results.—The results of the investigation are as follows:

Non-syphilitic Cases.—The 30 normal individuals in this group were recruited chiefly from medical students and physicians of The Johns Hopkins Medical School and Hospital. In all of them syphilis can be excluded. Seventy-seven patients included here were affected with 44 various non-syphilitic diseases and in these also syphilis could be largely excluded, although in a few instances they had not been questioned for a history of syphilis and the examinations were not complete. In all of the 30 sera from normal individuals, the Wassermann was negative with both antigens by both methods of fixation, as it was also in 74 of the 77 non-syphilitic patients. Of the three exceptions one, a patient in whom syphilis had not been specifically searched for, gave positive tests in both series with both antigens. This patient is to be regarded as undoubtedly syphilitic on the basis of the water-bath test. Another patient, whose clinical diagnosis was achylia gastrica, gave a history of gonorrhea but not of syphilis, and the Wassermanns were negative with both antigens in the water-bath, but positive with the cholesterinized antigen only in the ice-box. The third case was one of exophthalmic goitre, in which there was no anamnestic or clinical data regarding syphilis, but the Wassermann, which was negative with both antigens in the water-bath, was positive with both in the ice-box.

TABLE I. COMPARATIVE WATER-BATH AND ICE-BOX WASSERMANNS IN NON-SYPHILITIC PATIENTS

	Total cases	Wassermann tests*				Percentage of complete agreement of tests
		0000	4444	0044	0040	
Normal individuals..	30	30	..	..	..	100.0
Various diseases.....	77	74	1	1	1	96.1
Total.............	107	104	1	1	1	98.1

*In this and the following table, results of the Wassermanns are expressed numerically, from 0 (negative) to 4 (positive), as indicated in the text. The four figures at the top of each column represent in order the results of the test with cholesterinized and plain antigen in the water-bath, and with cholesterinized and plain antigen in the ice-box. Thus, 0000 and 4444 indicate respectively all tests negative or all positive; while 0044 indicates both tests negative in the water-bath and both positive in the ice-box, etc.

Thus, of 107 presumably non-syphilitic individuals, syphilis was surely present in 1, or 0.9 per cent, and possibly present in 2 more, 1.8 per cent. The total percentage of 2.8 is sufficiently low to be well within the limits of expectation. We conclude, therefore, that in this group of patients the probability is that no false positive results were obtained, and that the ice-box method of fixation with either antigen is not too delicate.

Syphilis Doubtful.—Seventeen patients are classified in this group, four of them because of a doubtful Wassermann reaction in the water-bath, and the remainder because of the equivocal clinical diagnosis or of suggestive points in the history or examination. In two of the cases in which a doubtful water-bath Wassermann was obtained with the cholesterinized antigen only, the reaction was positive with both antigens in the ice-box. In two similar cases the ice-box reaction was anticomplementary with both antigens. In two cases the test was positive with both antigens in the ice-box though negative with both in the water-bath; while in another case a doubtful test was obtained only with the cholesterinized antigen in the ice-box. In 10 instances all Wassermanns were negative.

In all cases in which a positive or doubtful ice-box test was obtained, the clinical evidence available pointed to a diagnosis of probable syphilis, so that, in this group also, we feel that no false positive reactions occurred.

Syphilitic Cases.—This group is composed of 148 treated and 28 untreated patients. In 80 cases there was complete agreement with the two methods, with 28 positives and 52 negatives. Fifty-nine of the sera, which reacted negatively in the water-bath, were positive by ice-box incubation. In not a single instance was the ice-box test negative or doubtful when the warm incubation gave a positive. In 4 sera, however, the ice-box test was anticomplementary, two being negative and two positive in the water-bath. In the remaining 33 cases there were 2 ice-box tests with anomalous results in the plain extract reactions, and 31 instances of incomplete reactions in various combinations.

TABLE II. COMPARATIVE WATER-BATH AND ICE-BOX WASSERMANN TESTS IN SYPHILITIC PATIENTS

	Total cases	Wassermann tests									Percentage of complete agreement	Percentage ice-box pos. when water-bath neg.
		4444	0000	0044	0030 to 0042*	44aa†	00aa	4044 to 4244	1044 to 3044	Anomalies		
Treated....	148	14	50	33	20	2	2	13	13	1 (4040)	43.2	37.8
Untreated .	28	14	2	1	1	2	2	4	1	1 (4441)	57.1	10.7
Total	176	28	52	34	21	4	4	17	14	2	45.4	33.5

*Both antigens negative in water-bath: one or both in complete fixation in ice-box.
† "A" = anticomplementary.

Greater sensitiveness was exhibited by the ice-box method in 50 per cent of this entire group, but the most striking difference is seen in the greater persistence of positive reactions or some degree of fixation by this method in treated cases. In untreated cases this sensitiveness resulted in 10.7 per cent more positives, as compared with an increase of 37.8 per cent among treated cases. The plain extract in the ice-box gives

more frequent complete fixation than cholesterinized antigen in the water-bath.

The Value of the Ice-Box Test in the Treatment of Syphilis.—Since the foregoing has shown that both antigens in the ice-box secure a higher percentage of positive Wassermann reactions than either antigen in the water-bath, and that in our experience no definite false positive results were obtained, it might be expected that this modification would be of value as a control in the treatment of syphilis. So far as we have been able to determine, reference to this point is lacking in the literature.

That this is actually the case is illustrated by the curves of Chart I. For the preparation of these curves, 148 cases

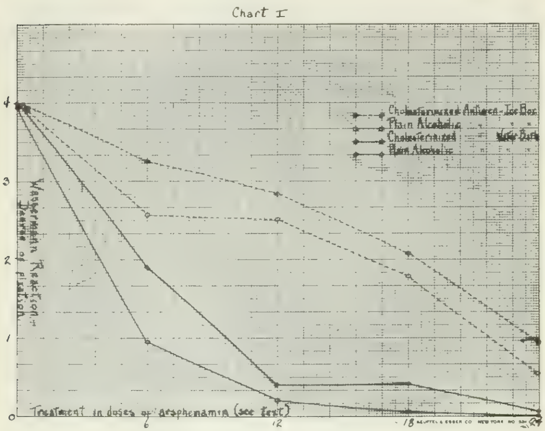

CHART I.—The Response of the Water-Bath and Ice-Box Wassermann to Treatment.

The composite Wassermanns of this chart were determined as follows: of the 148 treated patients, 61 had completed one course (6 doses) of arsphenamin. The four Wassermann tests on each of these patients were placed in vertical columns, and the numerals expressing the degree of fixation were added separately for each antigen, the sum total being then divided by the number of cases. This provides an average for the group which may be expressed as a definite fraction of the scale 0 to 4. By the same procedure, composite Wassermanns were determined at other periods during the course of treatment.

of treated syphilis, many of whom had had repeated tests by both methods, were available. Composite Wassermann reactions for each antigen were determined at arbitrary periods during the course of treatment, *i. e.,* before treatment, at the end of 6 doses of arsphenamin without mercury, and at the end of 2, 3, and 4 courses (each of 6 doses) of arsphenamin plus interim mercury. At the beginning of treatment, all Wassermanns are positive. By the end of the first course of arsphenamin, all have begun to drop toward negative, but not with the same degree of promptness. At this time, the composite test with the plain antigen in the water-bath is suggestive negative, with the cholesterinized antigen in the water-bath doubtful, while with both antigens in the ice-box it is suggestive positive. By the end of two courses of treatment, the discrepancy is even more striking. Both water-bath tests are now almost completely negative, though in the ice-box both are still of almost as great intensity as after the first course. Even after 24 doses of arsphenamin plus mercury, both ice-box tests still show some degree of fixation. The curves further provide an excellent demonstration of the relative sensitiveness of the plain and cholesterinized antigens by the two methods of preliminary fixation.

The number of cases on which these curves are based is too small to permit of final interpretation of the results. It is intended only to show that in treated syphilis the Wassermann reaction becomes negative much more slowly by the ice-box than by the water-bath method. Indeed, we have obtained in several patients, who had serologically fulfilled our requirements for the cessation of treatment with many repeated negative water-bath Wassermanns for more than a year, positive tests with both antigens in the ice-box.

These results indicate that our present standards of treatment of syphilis are probably too low, and that treatment should be continued over a much longer period of time than is now the case. The proper therapeutic control must in future include repeated Wassermann reactions by the ice-box as well as by the water-bath method. Further studies which are now being undertaken will provide more accurate data for the various types of syphilis, and will indicate, roughly at least, to what extent our present period of active treatment should be lengthened.

Discussion and Summary

In presenting this study we wish to express our realization of the danger attached to modifications of the Wassermann test which tend to increase its delicacy unwisely. The mechanism by which this is accomplished at low temperature incubation is not wholly understood. If it is due to deterioration of complement in some manner dependent on factors not connected with changes in the patients' serum resulting from the disease process, its usefulness as a diagnostic procedure is vitiated. Modifications and technical fads have done much to discredit the Wassermann reaction. Incredible errors occur daily from such practices with the most distressing consequences. An erroneous diagnosis of syphilis based on a nonspecific positive Wassermann, even when subsequently corrected, leaves a trail often impossible to obliterate. A negative Wassermann resulting from technical error leaves the door open for a future diagnosis; but a false positive, particularly if followed by antisyphilitic treatment, shuts out this possibility indefinitely, if not forever.

The study which we have presented is intended to demonstrate the results of the ice-box method in cases well studied and not dependent for diagnosis on this method. Although the series is small for such a purpose, it has demonstrated to our satisfaction an important superiority in its selection of uncured treated syphilitics. Although we favor its employment for diagnosis, as yet we do so guardedly, and accept no positive results without careful scrutiny of all available data in the case.

THE INABILITY OF STAPHYLOCOCCI TO FORM INDOLE FROM PROTEIN, PEPTONE AND TRYPTOPHANE*

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Since Jordan's[1] discovery that *B. influenzæ* produces indole, attention has been directed to the other bacterial invaders of the respiratory tract which might have this function. Among these microorganisms, the staphylococci are the only ones about whose ability to produce indole there has been any disturbing uncertainty. This doubt, for example, moderated the conclusions which Jordan drew as to the utility of his discovery, and delayed the publication of the report by Rivers[2] on the value of the test for indole in the spinal fluid as an aid in the diagnosis of meningitis due to certain strains of Pfeiffer's bacillus. In order to eliminate these doubts, and in order to assist the studies on bacterial metabolism, the decomposition of protein, peptone and tryptophane by staphylococci was investigated.

On inquiry among bacteriologists, we found the opinion generally held that staphylococci produce indole. Many, however, informed us that they had never seen a positive test for indole with a culture of staphylococcus. Evidently, their opinion was based upon the unequivocal statement in the usual text-books of bacteriology, for example, Park and Williams[3] and Hiss and Zinsser,[4] where it is asserted that staphylococci produce indole in peptone water.

A careful search through the literature revealed only a scant foundation for the broad statements which appear in the text-books. The first positive evidence that certain staphylococci may produce indole was supplied by Emmerling[5] in 1896. He planted *Staphylococcus aureus* in white of egg, which he states was sterile at first and contained no contaminating bacteria during the course of the experiment. After inoculation with the staphylococcus, the egg-white was incubated under anaërobic conditions for 14 days at 37°C. Gas was formed during the growth of the culture, and at the end of the experiment indole and skatole were recognized in the distillate of the fluid. The inadequate bacteriological controls of this medium in the preparation of which there is unusual opportunity for bacterial contamination, and the unique observation of the formation of gas from the proteins of egg by *Staphylococcus*, make it seem probable that the conclusion that the indole was produced by the staphylococcus may be erroneous. In 1902, Tissier and Martelly[6] isolated from putrid meat a strain of *Staphylococcus albus* which formed indole in a medium containing fibrin. Tissier's experience seems to have been the basis for the statements of Metchnikoff, whose writings gave vogue to the opinion that staphylococci form indole. In a paper on the intestinal flora, published in 1910, Metchnikoff[7] writes: "On sait—qu'un certain nombre de microbes de la flore intestinale de l'homme produisent de l'indole. Les staphylococques et le proteus, qui tous les deux se rencontrent souvent dans la flore intestinale sont aussi de producteurs de cette substance." We have not been able to find any data in the writings of Metchnikoff to support the contention just quoted. In discussing this question, Neisser[8] remarks that this statement by Metchnikoff must be an error. Since then, the only positive report which we have found occurs in the paper by Distaso[9] in 1912, in which he described *Staphylococcus asaccharolyticus*. This was a peculiar coccus, isolated from feces. It was about twice the size of the usual staphylococcus, grew only anaërobically, had no action upon the sugars and formed indole. A review of this evidence shows that the data are insufficient to warrant the statement that indole is a usual product of the metabolism of *Staphylococcus*.

In contrast to these few questionable reports, there are a number of papers dealing with the cultural characteristics of *Staphylococcus* which present a considerable volume of negative evidence on the results of indole tests with these microorganisms. The investigators who have been unable to find indole in cultures of *Staphylococcus* are: Buard,[10] Selter,[11] Dobrowski,[12] Müller,[13] Distaso,[14] Neisser,[8] Zipfel,[15] Honing,[16] Herzfeld and Klinger,[17] Winslow, Rothberg and Parsons.[18] The number of strains of various types of staphylococci, whose failure to produce indole is recorded in these papers, is approximately 300.

SOURCES AND CHARACTERISTICS OF STRAINS STUDIED

Our report on the action of *Staphylococcus* upon media suitable for the production of indole is based upon the study of 115 strains of this organism. These strains were isolated from pathological lesions in man, human feces, air or dust and from putrid beef, as shown in the following table.

TABLE I

Source	Number of strains
Furunculosis	8
Abscesses	12
Infected wounds and ulcers	64
Osteomyelitis	2
Adenitis	3
Septicemia	3
Peritonitis	3
Conjunctivitis	3
Throat or sputum	5
Feces	7
Air or dust	4
Putrid beef	1
Total	115

All of these organisms were Gram-positive, aërobic, facultative anaërobic cocci, and grew in the usual clusters on solid

* Presented at the meeting of the Society of American Bacteriologists, at Chicago, December 28, 1920. Abstract published in *Abstracts of Bacteriology*, 1921, V. 3.

media. On Loeffler's serum, 91 strains produced a yellow or orange pigment; 24 strains were white. All reduced nitrates to nitrites. Although groupings of staphylococci on the basis of cultural reactions have not permitted a strict or helpful classification, we studied for comparison their fermentation of glucose, lactose, sucrose and mannit, and their effect upon gelatin. When these data were compiled, according to the classification suggested by Winslow, Rothberg and Parsons,[18] we found that we had representatives of all the groups noted by them and in addition three strains for which their system makes no provision. The distribution of our strains in these groups was as follows:

A. Orange pigment:
   (a) Lactose fermented, gelatin liquefied. 89 strains. (*St. aureus.*)
   (b) Lactose not fermented, gelatin not liquefied. 2 strains. (*St. aurantiacus.*)

B. White pigment:
   (a) Lactose fermented, gelatin liquefied. 14 strains (*St. epidermidis.*)
   (b) Lactose fermented, gelatin not liquefied. 6 strains. (*St. candidus.*)
   (c) Lactose not fermented, gelatin not liquefied. 1 strain. (*St. candicans.*)

The other group not included in this scheme was composed of 3 strains of white staphylococci which did not ferment lactose but liquefied gelatin.

Tests for Indole

A variety of media were used for the cultures of staphylococci on which tests were made for indole. These were 1 and 10 per cent solutions of two brands of commercial peptone, a tryptic digest of casein, egg-white, and a solution in which tryptophane was the only nitrogenous substance. Before inoculation, all of these media were free from indole, and qualitative tests with the Hopkins-Cole glyoxylic acid reagent showed that they contained tryptophane. Colon bacilli readily formed indole when growing in these solutions.

Our experience with one brand of commercial peptone indicates the necessity of rigorous preliminary tests for indole in this material before its use for cultures in which a search is to be made for this derivative of tryptophane. A 1 per cent solution of this peptone sometimes gave a faint pink or rose color with Ehrlich's reagent. When this peptone was extracted with ether in a Soxhlet apparatus, the extract had a faint odor of indole and gave a deep rose color with Ehrlich's reagent. Furthermore, the distillate of the alkaline watery solution of this peptone, according to the method of Zoller,[19] also gave a strong indole-test with Ehrlich's reagent. No attempt was made to isolate indole from this peptone, but its use was discontinued in favor of a preparation of peptone which did not give the qualitative test for indole.

All strains were seeded in these originally indole-free media and incubated aërobically at 37°C., except the series of cultures in the solution of white of egg. In this experiment, the solution of egg-white was sterilized by filtration through a No. 12 Mandler filter, tubed, incubated for 48 hours to prove its sterility, and then inoculated with all strains of the staphylococci. The tubes were placed in a vacuum jar, from which the air was removed and partially replaced by hydrogen. The anaërobic cultures were then incubated for 3 weeks at 37°C.

Tests were made with the ether extracts of fluid cultures and with the distillates of some of the cultures in peptone water. The Ehrlich reagent was used as a routine, supplemented frequently by other tests, which, however, did not equal the Ehrlich test in delicacy or reliability. This reagent was prepared as follows:

Paradimethylaminobenzaldehyde	4 grams
95 per cent alcohol	380 c. c.
HCl (conc.)	80 c. c.

In performing the test for indole, the fluid culture was shaken up with ether, and Ehrlich's reagent added so that it spread as a layer between the ether and the peptone water or other fluid. A rose color, appearing in a few seconds first in the lower part of the ether extract, indicated the presence of indole. This test has been shown to be sensitive to 1 part of indole in 1,000,000 parts of fluid.[20]

Results of Tests for Indole

Tests for indole upon the cultures of staphylococci in these media were made at intervals of 48 hours, 96 hours, 7 days and 14 days. The organisms grew abundantly in the peptone solutions and the medium made with the tryptic digest of casein. Their growth in the solution of egg-white was very scanty. The growth in the solution of tryptophane will be described in detail in a subsequent paragraph. The results of all the tests for indole upon the cultures of the 115 strains of *Staphylococcus* in these media under the conditions described above can be stated briefly: they were all negative. No evidence was obtained that any of these varied staphylococci produced even a trace of indole in media containing free or combined tryptophane.

Cultures in Solutions of Tryptophane

Zipfel[15] has pointed out that the critical test of the ability of an organism to produce indole is its cultivation in a medium containing tryptophane. The solution used by him contained asparagin and ammonium lactate in addition to tryptophane. The 8 strains of *Staphylococcus* studied by him failed to produce indole in this medium. As it is possible that the staphylococci derived their nitrogen from the asparagin and ammonium lactate, leaving the tryptophane intact, we limited the conditions more narrowly in our experiment by using a solution in which tryptophane was the only source of nitrogen. If the organism multiplied in this medium, it would be forced to obtain its nitrogen from tryptophane, the substance from which indole is formed. In the utilization of tryptophane, the organism would presumably derive nitrogen from the NH_2 group of the α-aminopropionic acid attached to the indole nucleus, as this nitrogen atom is more readily split off than the N of the pyrrole ring. In this decomposition, it is possible that the tryptophane might be deamidized only to indole propionic acid, or might be reduced further to indole. To determine which of these processes occurred in the decomposition of

tryptophane by *Staphylococcus*, the following medium was prepared:

Tryptophane (pure)	0.5 gm.
Na_2HPO_3	0.2 gm.
NaCl	0.5 gm.
$CaCl_2$	0.02 gm.
$NaHCO_3$	0.02 gm.
Distilled water	100 c. c.

This solution was sterilized by filtration through a No. 12 Mandler candle. No precipitate occurred when it was sterilized by filtration. If, however, the liquid were boiled a precipitate appeared, due probably to the formation of calcium phosphate. The reaction was Ph 7.4-7.6, without adjustment. After filtration, the flasks and tubes of media were incubated at 37° C. for 48 hours to prove their sterility. In one experiment, 0.5 per cent glucose was added to the solution. Tests for indole and indole propionic acid with this medium before inoculation were negative. *B. coli* grew readily in it, and produced indole within 8 hours.

Tubes of the tryptophane solution were inoculated with a loop of each strain of our series of staphylococci from a 24-hour agar slant culture. This made the small amount of fluid, 2 c. c., in each tube slightly turbid. In some, the turbidity increased after 24 hours and a granular sediment of organisms appeared after 4 days. At the end of 7 days, the usual tests for indole were made with each culture. These were negative.

As not all strains grew equally well in this medium, several were selected because of their more abundant growth for more detailed experiments. With them, bacterial counts were made by the dilution and plating method to show that the organisms multiplied in the tryptophane medium. Amino nitrogen and total nitrogen determinations by the Van Slyke and Kjehldahl methods were made for us by Dr. D. Wright Wilson upon the original solutions and the solutions in which the staphylococci had been cultivated. The usual qualitative tests for indole, particularly Ehrlich's test, and the ferric chloride test for indole propionic acid were applied to this medium both before its inoculation and after the growth of the staphylococci. A summary of such an experiment is shown in Table II.

This experiment demonstrated that *Staphylococcus* grew in this solution of tryptophane, that as a result of the metabolism of the coccus the tryptophane lost 17 per cent of its amino nitrogen. Indole was not formed at any period during three weeks. The solution became slightly less alkaline. The doubtful tests with ferric chloride might indicate the formation of indole propionic acid. The chemical studies show definitely that tryptophane is deamidized by *Staphylococcus*, but as yet are insufficient to prove that indole propionic acid is actually one of the substances formed in this decomposition.

SUMMARY AND CONCLUSIONS

In order to test the ability of staphylococci to produce indole, 115 strains of *Staphylococcus*, isolated from pathological lesions in man, from air, dust, human feces and putrid beef, were cultivated in media containing proteins, peptone and free tryptophane. The conditions under which the cultures were incubated at 37°C. were both aërobic and anaërobic. Numerous tests for indole with several reagents, particularly with Ehrlich's paradimethylaminobenzaldehyde solution, were made with the cultures at different intervals from 48 hours to 3 weeks. In no instance was a positive test for indole obtained. Particular significance is attached to the negative result of tests for indole with cultures of staphylococci in a fluid in which pure tryptophane was the only source of nitrogen, aside from the air, available for the growth of the organisms.

Although these results present only negative evidence, they are thought to be sufficient to warrant the conclusion that indole is not a product of the metabolism of *Staphylococcus*.

TABLE II. STAPHYLOCOCCUS ALBUS NO. 115 IN 0.5 PER CENT TRYPTOPHANE

Hours after seeding	No. bacteria per 1 c.c.	Chemical analysis.
		Before inoculation. Ph. 7.6. Indole = 0. Indole propionic acid = 0. Total N = 0.522 gm. per 100 c. c. Amino N = 0.292 gm. per 100 c. c.
0	28,800,000	
1.5	29,400,000	
3	19,200,000	
4	25,600,000	
5	56,000,000	
6	144,000,000	
7	192,000,000	
8	320,000,000	
12	416,000,000	
14	410,000,000	
22.5	380,000,000	
		After 2 weeks. Bacteria removed by centrifuging at 2500 r. p. m. for 1 hour. Ph. 7.4 (approx. colorimetric). Indole = 0. White ppt. with Fe_2Cl_6 which turned red on heating. Possibly indole propionic acid? Total N = approximately same. Amino N = 0.242 gm. per 100 c. c.

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HEMOLYTIC EXUDATES AND TRANSMISSIBLE BACTERIAL AUTOLYSIS[1]

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What I shall say to you today cannot properly be termed a lecture; but rather a short communication bearing on some quite recently discovered experimental facts.

Among the fundamental and characteristic properties of life, the most remarkable undoubtedly are reproduction, which creates a new being, and heredity, thanks to which this new being closely resembles its parents. In fact, heredity consists in the transmission of variations, which, conferring new qualities to certain beings, gives rise to special and readily recognizable types and in this way allows of an easy discrimination between the different species, varieties, and families. What then is the intimate nature of the mechanism through which a given variation, occurring at a certain moment, may be transmitted to the offspring?

It is hardly necessary to say that every variation must perforce depend upon an immediate factor, capable of determining it directly in the cell, and which itself has been called forth by an external influence. When this external influence is permanently exerted while the cell multiplies, thus constantly causing the factor of variation to be present, it is easily conceivable that the variation itself may be conveyed from one generation to the following one, since the primary influence acts without interruption. But when the external influence is only a transient and evanescent one, disappearing at once after having brought into action the factor of variation, the transmission of the modification thus created is not so readily accounted for. Such being the case, it must necessarily be accepted that the variation could not be bequeathed through the successive generations, unless in every one of the successively engendered individuals the factor of the aforesaid variation is regularly renewed or created again and again, so as not to lose its original power and primary efficiency. In fact, were this factor not reproduced while being transmitted, of a certainty its strength would be very soon exhausted, as an unavoidable consequence of its being distributed or, if I may say so, diluted among the more and more numerous representatives of an ever-increasing offspring. Unless constantly regenerated, this factor would very soon be weakened to such an extent as to become wholly incapable of unceasingly compelling the new cells, derived from the repeated divisions, to assume the special features and aspect which for the first time appeared under its influence. It follows, then, that at the end of a limited period, the variation itself could not be transmitted any more, the factor which commands it becoming more and more attenuated as a consequence of its being progressively more and more widely distributed. As an analogy, one might say that a railway track can guide an engine to the end of its journey, precisely because it offers the same resistance and solidity all along the way, thus constantly playing an active part, that is, unceasingly repressing any tendency to deviation. Similarly, when an initial cell has been primarily modified through the agency of an external and temporary cause, the regular and henceforth everlasting transmission of the induced modification absolutely requires that the immediate factor of the latter, once created, be regularly regenerated while acting and being bequeathed.

Let us now suppose that the cell under consideration is a microörganism, and furthermore, that the direct factor of variation is an active principle which this microörganism has produced under the influence of a temporary external cause, such as a special and exceptional composition of the nutrient medium wherein it has lived during a certain period of time. Following this line of thought, we immediately anticipate that the microbe in which the variation due to this active principle has primarily occurred, will, while multiplying, bequeath to its offspring the capacity of elaborating the same substance, the indefinite persistence of the aforesaid modifications being thus insured and maintained *ipso facto* through the successive cultures. Let us imagine, moreover, that the principle alluded to is diffusible in the liquid culture medium. Such being the case, the variation now may be not only hereditary but also contagious, since the liquid in which the modified bacterium has lived contains precisely the agent capable of impressing the same modification on identical but still normal and unaltered microörganisms. These latter will be thus similarly changed, and will, moreover acquire the property of transmitting the same modification to their own offspring, or merely by means of contact to normal bacteria, and so on indefinitely. If we finally assume that the variation consists in a disturbance of nutrition, namely, in a disturbance of the equilibrium between the building of living matter and its destruction, the latter process being exaggerated, then the transmissible modification will be characterized by the fact that the organism thus changed will, henceforth, show a marked tendency to autolytic phenomena.

These views seem to be merely theoretical, but we are entitled to transfer them in their entirety into the sphere of positive and real things, of unquestionable experimental results, as I now shall try to show. As a matter of fact, they may be brought into complete harmony with experimental facts connected with the problems of immunity, which have been observed by myself and my friend and co-worker, Ciuca, and to which I shall briefly refer.

[1] Lecture III of the Herter Series, delivered before The Johns Hopkins University on Thursday, October 28, 1920.

A guinea-pig receives into the peritoneal cavity, at intervals of five or six days, two or three injections of a liquid culture belonging to the group of B. coli. Twenty-four or forty-eight hours after the last injection, the cavity is punctured and a few drops of the leucocytic peritoneal exudate are extracted. Now, this exudate possesses, as may be shown simply by adding to it a small amount of culture, the property of modifying the same colon bacillus in such a manner that it confers upon it a highly marked and henceforth transmissible autolytic power. The conflict between the leucocytic exudate and the bacterium induces the latter to elaborate a lytic substance, which, added to a normal turbid broth culture, clarifies it almost completely in the space of a few hours. Now, one droplet, or even less, of such a clarified culture, transferred into a second broth culture, normal and turbid as the first, develops in it the same process of clarification; subsequently, the second culture thus treated acts in the same way towards a third one, and so on. The experiment may be repeated again and again, one condition, however, being absolutely necessary; no transmission takes place in killed emulsions, *the culture must be living,* and if this requirement is complied with, the active principle responsible for the phenomenon is unceasingly reproduced. But, as it is found to resist a temperature of about 60-65°C., which kills the bacterium, one observes that a broth culture which has been subjected to the lytic process, and subsequently sterilized, is still able to induce the lytic influence in a living normal culture, which in turn becomes capable of transmitting it to another identical culture, and so on, as has just been told.

The technical detail is quite simple. The peritoneal exudate wherein phagocytosis has occurred, but which nevertheless still contains some living germs, is added to two or three volumes of sterile broth, the fluid thus obtained being then heated at 58°C., for nearly half an hour. Now, more broth and a droplet of living broth culture of B. coli having been introduced, the mixture is incubated. The development of the microörganism is rather difficult; only a slight turbidity appears. After having been maintained in the incubator for nearly twenty hours, the mixture is kept at the room temperature for two or three days, during which time the liquid does not become more turbid, but on the contrary shows rather a tendency to clarification. Now, when it is heated again at 58°C., it is found that a few drops of such a fluid, added to a tube of sterile ordinary broth, make the latter unfit for the growth of the colon bacillus; the nutritive medium keeps clear after being impregnated and incubated; the development is entirely hindered. On the other hand, if a few drops are introduced into a fresh and already turbid colon broth culture, clarification takes place. When the lysis has fully occurred, this culture, whether sterilized at 58°C., or not, has *ipso facto* become capable of inducing the same phenomenon in a new identical broth culture and so on, the lysogenic property being regularly and forever transmitted to the later generation of bacteria.

When a normal broth culture, already sufficiently developed, that is, showing a marked turbidity, is subjected to the clarifying influences just mentioned, it is not, in fact, completely sterilized, a certain proportion, but in reality a rather small proportion, of the bacteria which are present seem not to be affected and are still living. However, they do not grow easily in broth; when they are transplanted into a new tube of this nutrient medium, the latter becomes merely opalescent in the incubator, but—and this is a very important fact—acquires the lytic quality. Difficult in broth, the development of these germs is considerably easier and abundant on agar. After some transplantations a very rich agar culture may be obtained, the aspect of which, however, is strikingly different from that of the culture on agar of the normal unaltered B. coli. Instead of being bluish and rather thin and translucent, the agar culture of the colon bacillus which has withstood the lysis, shows an opaque, whitish and thick layer which is remarkably mucous and even semi-fluid. It is a surprising fact that this modified culture, which in reality represents a new variety, keeps permanently, even when repeatedly transplanted, the property of communicating to sterile broth, when added to it even in infinitesimal amount, the lytic character. Hence it is allowable to conclude that henceforth and forever the modified bacterium carries the autolytic property, unceasingly regenerating it while growing. It is worth mentioning that the colon bacillus transformed in that way shows also some changes in other respects. It has kept its fermentative and indole producing properties, but is no longer capable of acting on neutral broth. Its virulence, instead of having been reduced, is visibly increased. It is not so easily phagocytable as the normal original bacterium; when inoculated in sufficient amount into the peritoneal cavity of a guinea-pig it passes into the blood of the heart, always carrying its characteristic, henceforth inseparable and permanent, lytic capacity.

The phenomena just described must now be carefully confronted with the results observed by the French writer d'Herelle, which, according to this scientist, find their explanation in the presence of a living, invisible virus, the so-called "bacteriophage."[2]

In several interesting papers which have been published since 1917 in the Comptes rendus de la Société de Biologie or in the Compte rendus de l'Académie des Sciences, d'Herelle pointed out that when a mixture of broth and fæces from patients recovering from dysentery is incubated for about twenty hours and subsequently filtered, a clear fluid is obtained, possessing the wholly unexpected property of clarifying a thick suspension of dysenteric bacilli. Furthermore, a trace of such a suspension which has thus been rendered clear, added to a second suspension of the same microörganism, brings about the same effect, and so on. Similar results have been obtained by d'Herelle for other intestinal microorganisms, provided, however, that the fæces came from individuals who had recently suffered from a disease of the digestive tract or who had been associated with such patients. For example, d'Herelle obtained fluids exerting a lytic influence on

[2] We found recently that the British scientist Twort published in the *Lancet* (1915), two years before d'Herelle, a most remarkable paper in which quite analogous facts are mentioned.

a microörganism belonging to the group of the colon bacillus which was precisely the one employed in our experiments just recorded.

D'Herelle explained his findings by admitting that the lysis was due to a filterable, that is, an extremely finely divided virus, capable of living as a parasite in the inside of several bacteria such as dysentery bacilli. This virus would multiply in the bacterial bodies whenever conveyed into a new suspension. D'Herelle succeeded in demonstrating that the regeneration of the lytic agent, supposed to be a living one, did not occur in sterile broth nor in suspensions of organisms which had been previously killed. Under such circumstances, the lytic power is very soon exhausted when its transmission from tube to tube is attempted.

The experimental facts discovered by d'Herelle are unquestionable, but the explanation this writer has thought proper to offer is not correct. Objections were recently raised against it by Kabeshima, who observed that the lytic power shown by clarified bacterial suspensions obtained according to d'Herelle's indications could still be elicited and transmitted to new living suspensions even when the active fluids had been heated at about 70° C., or treated with substances unquestionably possessing antiseptic properties, such as acetone, ether or sodium fluoride.

Taking into account the well known fact that dysenteric fæces are very rich in leucocytes, and that according to d'Herelle's observations the lysogenetic property of such fæces appear only at the period of convalescence, we felt free to imagine that perhaps the lytic phenomenon observed was merely the consequence of some immunological process, or, to speak more precisely, was the result of a special modifying influence exerted by the leucocytes on the microörganisms. According to this hypothesis, the conflict between the leucocytes and the bacteria would result in inducing an hereditary vitiation of the bacterial metabolism, consisting chiefly in the production by the microörganism itself of some autolytic ferment which diffuses into the surrounding medium and consequently acts in the same way on normal bacteria belonging to the same species, when these are added to the active fluids. As I told you some minutes ago, our supposition has been fully confirmed by adequate investigation. In reality, the lytic property may, without any interference on the part of the intestinal tract or of its germs, appear in a mixture of culture and of a leucocytic inflammatory exudate containing no other living microörganism. The hypothesis advocated by d'Herelle based on the presence of a very small parasite growing inside the bacteria, if it had proved true, would indeed have been suggestive, but the correct explanation afforded by our experiments seems to be, as regards the biological significance, of still wider interest, since it offers a striking example of a nutritive modification which, once impressed through the agency of the defensive factors of the inoculated animal, is henceforth maintained and reproduced forever in the successive generations, as I emphasized above.

Whatever the method made use of to elicit the lytic property, be it the technique of d'Herelle based on the activity of the fæces of convalescents, or the experiment we performed by bringing into contact a leucocytic exudate and the bacterium, the study of the chief peculiarities of the phenomenon gives quite identical results. For example, the agar culture of the modified colon bacillus which, as I pointed out above, definitely behaves as the carrier of the lytic influence, may be obtained just as well and with the same characteristics by employing either the fluid of d'Herelle derived from the intestinal tract, or the lytic mixture which we obtained by means of a leucocytic exudate. Whatever the manner in which the active principle has appeared, its properties may be demonstrated by adding it either to an already grown broth colon bacillus culture, in which case its influence is shown by the supervening clarification, or to sterile broth containing a droplet of fresh normal colon bacillus culture, in which case the bacterial multiplication is altogether prevented, the initial fluid preserving for several days, when incubated, its original limpidity.

Another very simple and particularly striking method of detecting the lytic power consists in depositing a droplet of the lytic fluid on the surface of an incubated nutrient agar on which, living colon bacilli having been spread some hours before, a thin bacterial layer is just beginning to appear. The tube is subsequently kept in the incubator, the layer soon thickens over the whole surface, except at the points which have come in contact with the lytic droplet, growth here having been altogether inhibited and the agar surface remaining bare as before. After two or three days, however, a few colonies appear on this bare spot, consisting of resistant organisms, subsequently capable of growing abundantly when transplanted upon a fresh agar tube, assuming the special aspect described above, and preserving permanently, while unceasingly growing, the lysogenetic property.

There is no need to say that we are now going on with the experiments in order to ascertain whether analogous lytic phenomena may be, by means of our technique based on the production of leucocytic exudates, observed with several other microörganisms, belonging to saprophytic or pathogenic species. At any rate, it appears to us to be highly desirable that similar investigations be likewise carried on in other laboratories, since the question of the transmissible power of autolysis in bacteria seems worthy of the attention of the bacteriologist as well as of the biologist.

PREGNANCY FOLLOWING IMPLANTATION OF THE OUTER END OF THE ONLY REMAINING FALLOPIAN TUBE INTO THE UTERINE CORNU AFTER RESECTION OF A CORNUAL PREGNANCY

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I am reporting this case not because it is one of cornual or interstitial pregnancy, although these conditions are by no means common, but because as a result of a rather unusual operative procedure the possibility of conception was preserved in a case in which at a previous operation the right tube and ovary had been removed and in which at the second operation a cornual pregnancy was found on the left side. For my own part, I should not have thought of saving the outer part of the left tube, but Dr. Cullen laid it carefully aside and, after removing the cornu, inserted the proximal end of the remaining portion of the left tube into the uterine cavity. Pregnancy followed.

In the literature at my disposal I have been able to find the record of only one similar case,[1] although from time to time the inner end of the Fallopian tube has been inserted into the uterine cavity.

Gyn. No. 25283. F. L., aged 36, white, was admitted to The Johns Hopkins Hospital on October 3, 1919, complaining of irregular menstrual periods. The patient was a frail woman, weighing only 113 pounds. Her family history was good. She had had typhoid fever and malaria.

The patient had been married six years. She had had one child who had died at two years of age, and two miscarriages, one nine months after marriage at three months, and the other in March, 1919, at two and a half months.

Her last regular period had been in March. For seven weeks following this there had been no bleeding at all. After this there was a period which lasted eight days. She apparently had had a regular period four weeks before admission. A few days before the patient entered the hospital bleeding had reappeared and there has been irregular bleeding since that time. No large clots had been passed and no membranes had been noticed, but the bleeding had increased in amount.

On examination a midline scar was found below the umbilicus. There was little or no relaxation of the outlet. The cervix was lacerated. The body of the uterus was apparently normal in size and in good position. At the left cornu, however, was a rounded mass several centimeters in diameter. This appeared to be adherent to or blended with the left cornu. It was not tender and and felt rather soft. It was fairly movable. The general picture suggested a left tubal pregnancy.

Operation Oct. 6, 1919, by Dr. Cullen. The old abdominal scar was excised. On opening the peritoneal cavity an adhesion was found between the upper surface of the uterus and the lower end of the scar. This adhesion was clamped, cut and tied.[2]

In the left uterine horn we found a tumor approximately 5 x 4 cm. (Fig. 1). On the median side this blended with the surface of the uterus and on the outer side was continuous with the tube. There were enlarged blood vessels running over its surface. Undoubtedly we had to deal with a cornual or interstitial pregnancy.

Dr. Cullen temporarily clamped the tube at its cornual attachment and drew it to one side. He then resected the uterine horn leaving a raw area approximately 4 x 2 cm. at its uterine attachment. The Fallopian tube was now drawn into the uterine cornu so that its inner end lay in the cavity of the uterus (Fig. 2). The wound was then approximated. The excess of bladder peritoneum was drawn up over the raw area to prevent adhesions. After the operation was completed, the fimbriated end of the Fallopian tube, which lay free, was about 1 cm. long. One cigarette drain was placed in the lower angle of the abdominal incision and the wound closed.

The patient made a most satisfactory recovery and was discharged on October 23, 1919, just 17 days after the operation.

Gyn.-Path. No. 26637.—The growth at the left uterine cornu was 5 cm. long, 3.5 cm. broad, and projected 4 cm. from the surface (Fig. 3). It was perfectly smooth and had a delicate tracery of blood vessels covering its outer surface. Its basal attachment to the uterus was 4 x 2 cm. The walls of the sac on section varied from 1 to 3 mm. in thickness and the cavity was filled with blood (Fig. 4).

Sections through the cornual pregnancy show an outer covering of muscle varying from 1 to 3 or 4 mm. in thickness (Fig. 5). At no point does one find any evidence of an inner epithelial lining. The blood which fills the cavity extends right up to the muscle. At the junction there is some young connective-tissue formation. At some points are aggregations of polymorphonuclear leucocytes and small round cells. Here and there are perfectly free spherical cells that have taken up brown pigment. In the center of the blood-clot is an early placenta with an amniotic sac in its center. The chorionic villi are typical.

On Dec. 28, 1920, I received a letter from the patient saying:

I am getting along very nicely. I was threatened with an abortion at two months, but have been very careful since then, and have had no further trouble.

[1] Plastic Operation on the Fallopian Tubes, by Emil Ries, American Jour. Surgery and Gynecology, March, 1899, Vol. XI, p. 180.

Ries says: "Occlusion at the uterine end of the tube is produced quite frequently by small adenomyomas of the tube, but there is only one case on record where an operation was performed for such an occlusion. This operation was performed by Dr. T. J. Watkins of Chicago, and I gave the first short notice of it in my paper on Nodular Forms of Tubal Disease (Jour. Experimental Medicine, 1897). This operation practically amounts to an end to end anastomosis between the tube and the uterus—a salpingo-hystero-anastomosis. It was performed so that the myomatous nodule was excised by a wedge-shaped incision reaching down into the cavity of the uterus, the tube then being implanted into this opening and retained by sutures. The appendages on the other side had been removed on account of pyosalpinx. The patient miscarried a number of months after the operation, proving thereby that the salpingo-hystero-anastomosis had kept patent the communication between the tube and uterus."

[2] In 1918, at another hospital, the uterus had been suspended, and the right tube and ovary removed. The adhesion extending from the upper surface of the uterus was undoubtedly due to the old suspension.

On March 21, 1921, I received a letter from her saying:

Soon after writing you last I began having bearing down feelings. Dr. Porter had me go to bed and gave me something in case the pains began. I was in bed two weeks and finally lost my baby on the 11th of March. The baby was seven months or a little over. A fine child and well developed. Dr. Porter did everything he could to ward off labor but nothing did any good. The afterbirth came first and it was a foot presentation.

Dr. D. S. Porter of Andrews, S. C., this patient's family physician, under date of April 25, 1921, kindly sent me further particulars. He said:

I had to keep her in bed the greater part of the time and was under the impression that the pregnancy was further advanced than she claimed, as the uterus at the time of her confinement was on a level with the costal margin.

There was a marginal placenta prævia and a breech presentation. The baby was premature, I should judge about seven months. It was born dead.

In the Gynecological Department Dr. Cullen has for years been urging the utmost conservatism where pelvic inflammatory conditions exist during the child-bearing period, and wherever possible has preserved the menstrual function, feeling that in many cases the unsexing of the woman in early or early middle life was more deleterious to her than the thought or probability of an occasional second abdominal operation.

In this case the logical procedure seemed to be removal of the left tube with the cornual pregnancy, but the saving of the outer portion of the tube and its insertion into the uterine cavity proved well worth while.

The patient was most anxious to become pregnant again and had it not been for the placenta prævia she would in all probability have gone to term. We shall look forward with interest to the subsequent history of this patient as she is still under forty.

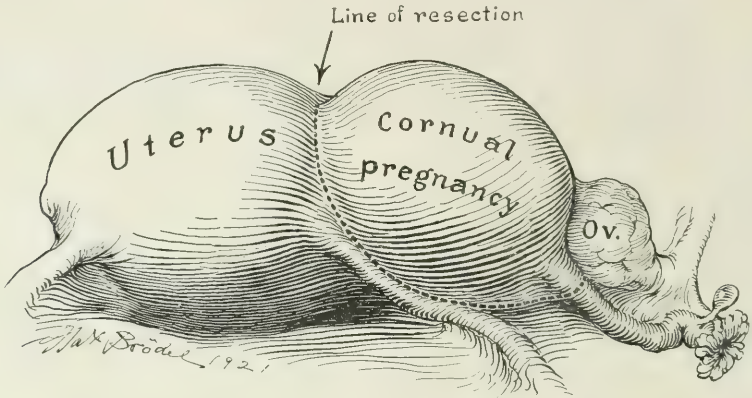

Fig. 1.—Resection of a Left Cornual Pregnancy.

Gyn. No. 25283. The right appendages had been removed at a previous operation. The left cornual pregnancy was cut away, as indicated by the dotted line. For the subsequent handling of the left tube see Fig. 2. For the appearance of the cornual pregnancy see Figs. 3, 4 and 5.

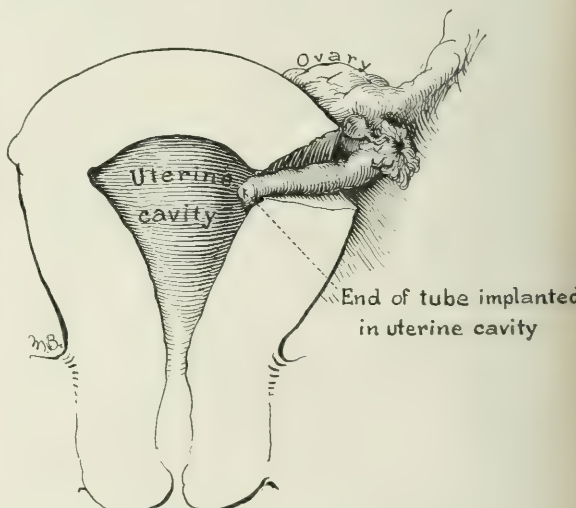

Fig. 2.—Implantation of the Fallopian Tube into the Uterine Horn.

Gyn. No. 25283. The right tube and apparently the right ovary had been removed elsewhere at a previous operation.

The outer end of the left tube seen in Fig. 1 was drawn into the uterine horn until its inner end lay in the uterine cavity. The cut edges of the uterine horn were now snugly approximated with catgut. About 1 cm. of the outer end of the tube then lay free in the abdomen. Pregnancy soon followed through this tube.

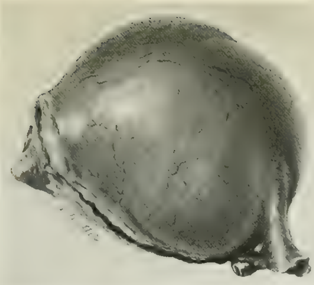

Fig. 3.—Pregnancy in the Left Uterine Horn.

Gyn. No. 25283. Gyn.-Path. No. 26637. The left and under surface represents the uterine attachment of the cornual pregnancy. A small piece of the left tube has been removed with the cornu. For the appearance on section see Fig. 4.

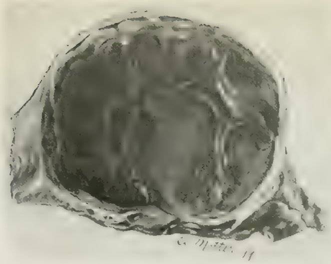

Fig. 4.—A Left Cornual Pregnancy.

Gyn. No. 25283. Gyn.-Path. No. 26637. This is a longitudinal section through Fig. 3. There is a thin outer wall of muscle. The greater part of the specimen consists of old blood. Fig. 5 shows that the central portion of the clot contains placental tissue.

Fig. 5.—Pregnancy in the Uterine Horn.

Gyn. No. 25283, Gyn.-Path. No. 26637. This is Fig. 4 enlarged five times. *a* is the outer muscular wall; *b* the blood-clot filling the cavity; *c* the amniotic sac containing some granular material. Extending out into the blood-clot are numerous placental villi, two of which are indicated by *d*.

NOTES ON NEW BOOKS

The Diseases of the Genital Organs of Domestic Animals. By W. L. WILLIAMS. Pp. 856. (Ithaca, N. Y., 1921.)

This large volume by the professor of obstetrics and of research in the diseases of breeding cattle in the Veterinary College of Cornell University is highly creditable to its author and represents a valuable contribution to American medical literature. I earnestly recommend it to scientifically minded obstetricians and gynecologists, as I feel sure that when they have read it they must agree with me that they have learned much, have had their imagination stimulated, and have to confess, so far at least as certain problems are concerned, that human gynecologists may learn something from their veterinary colleagues.

Space will not permit a complete review of this excellent work, and consequently I shall dwell upon only a few points which have particularly interested me. After a satisfactory account of the normal anatomy of the male and female generative organs of cattle, including a fairly accurate section upon the structure of the placenta, the author takes up the changes which occur in disease, and shows that in both sexes, but particularly in the female, many lesions occur with which we are familiar in human beings.

In considering the estrual cycle, the author is a firm believer in the regulatory function of the corpus luteum, and points out that estrus will not set in so long as the corpus luteum of the last period persists. Accordingly, whenever it is delayed, he advocates squeezing the old corpus luteum out of the ovary by pressure exerted through the rectum, which within three days is followed by the appearance of estrous symptoms. Indeed, he goes a step further, and states that whenever the induction of abortion is indicated in cows, it can be most readily effected by the same maneuver, which he holds is practically infallible and far more efficacious than the procedures formerly employed. Such observations go far toward establishing the Fraenkel-Born theory concerning the function of the corpus luteum, and appear difficult to reconcile with the fact that in women the removal of an ovary containing the corpus luteum does not bring about the termination of pregnancy after the first few months.

The removal of the corpus luteum by rectal manipulation naturally suggests the methods of gynecological diagnosis in the cow, of which rectal palpation is the most important. By its means it is possible to recognize not merely the existence of pregnancy as early as the end of the second month, or even to diagnosticate the presence of twins, but also to palpate the tubes and ovaries and to detect the presence of tumors, persistent corpora lutea or other abnormalities. Vaginal exploration is employed to a lesser extent, but what is said concerning the inspection of the cervix, as well as concerning intrauterine manipulations, is very suggestive of human gynecology.

To those unacquainted with veterinary medicine possibly the most interesting portion of the book is that dealing with venereal disease in domestic animals. Thus, the author describes the vesicular and the granular venereal diseases of cattle, which likewise occur in goats and swine; dourine and horse pox in horses; venereal granulomata and catarrhal venereal disease in dogs; and, finally, an inflammatory venereal disease which affects rabbits. All of these diseases are definitely due to coital infection.

Although only a few words can be said concerning certain of them, it will surprise most laymen to learn that 90 per cent of the cattle in this country are suffering from granular venereal disease, from which they rarely recover completely, as well as the fact that most other domestic animals are liable to venereal disease of some sort. Such knowledge will be regarded as a stumbling block by many "uplifters," and particularly by those who regard the existence of venereal disease as a specially devised punishment for sexual sin, and who regard any steps which may be advocated to bring about its abolition as tending to popularize sexual immorality. If they feel thus toward man, what must they say concerning the mild-eyed cow, which is almost universally infected, or concerning the harmless rabbit which frequently becomes infected at the time of mating?

It was a surprise to me to learn that dourine has been observed in horses in this country, and it will surprise many to be told that this very fatal disease is due to infection by a trypanosome, which, instead of being transmitted by the bite of an insect, is conveyed from animal to animal by sexual intercourse, and, furthermore, that its presence can be detected in the blood and spinal fluid of the infected animal. Likewise, it is interesting to learn that the venereal granulomata of dogs are thought to be due to a spirochete, which, however, bears no relation to that causing syphilis.

Certainly the most important section of the work, in the estimation of the author, is the one dealing with infections of the generative tract. The average medical man is vaguely aware that cows and other domestic animals occasionally suffer from puerperal infection, analogous to that observed in women, and that epidemic abortion may cause great economic losses to stock raisers, and is currently believed to be due to infection by a specific micro-organism, the bacillus abortus of Bang. Williams, however, believes that while this bacillus may sometimes lead to abortion, it is not the only organism concerned. On the other hand, he holds that its significance has been greatly over-estimated, and that the general acceptance of its specific character has served to obscure the issue, as well as to prevent the recognition of the fact that the problem is much more complicated than is generally believed. He states that abortion may be caused by any one of a number of micro-organisms, including the bacillus abortus, and that most cattle suffer from such infections before they become pregnant, so that the occurrence of abortion, dystocia, retention of the fœtal membranes or actual puerperal infection depends upon the virulence of the infection, as well as upon the resistance offered it by the infected animal. In this connection Williams holds that identical bacteria are concerned in the production of various inflammatory diseases of the male genitalia, so that coitus between healthy and infected animals is extremely dangerous for the normal partner, whether male or female, and that infection thus established constitutes a serious danger not only for the individual concerned, but for the entire herd.

Furthermore, the consequences of such infections are not limited to the genitalia of the animals involved, but in many instances the bacteria gain access to the fœtus in utero and cause its death, or lead to the birth of congenitally infected offspring. The mode of fœtal infection is of great interest. Except in the presence of the most virulent infection, the author believes that the placentomata of the cotyledonary placenta offer an efficient barrier against the transmission of bacteria from the mother to the fœtus. On the other hand, he assumes that the portions of the chorion between the cotyledons are very vulnerable to bacterial invasion and readily permit the passage of bacteria into the amniotic cavity, where they may be swallowed by the fœtus and lead to its infection. Evidence to this effect is afforded by the fact that the same bacteria can be found in the stomach and intestinal tract of the dead fœtus as in the maternal uterus, and that pellets of meconium containing similar bacteria are present in the liquor amnii. As the latter are normally absent, he attributes their presence to diarrhœa from which the fœtus had suffered while still *in utero*. Furthermore, in the case of animals which are born alive, the existence or the prompt development after birth of dysentery, pneumonia or pyæmia clearly points to intrauterine infection.

While Williams apparently makes out a clear case for the occurrence of congenital infections in cows and sheep, it is questionable whether they occur in human beings. Owing to the different structure of the bovine and human placenta, the relations existing between the uterine wall and the fœtal membranes differ greatly, so that what may hold good in the cow will not necessarily apply to human beings. It must, however, be remembered that the observations of Slemons upon the

development of placental bacteriæmia, in certain cases of intrapartum infection in women, make it possible that we may have overlooked a possible source of antenatal infection, and, consequently, Williams' observations should stimulate us to renewed investigations along such lines.

In cases of relative sterility and abortion the microscopical study of the anatomical structure and peculiarities of the spermatozoa of the bull are likewise very suggestive. Williams points out that in certain instances actively motile spermatozoa may present abnormalities, which, while not sufficient to interfere with impregnation, may so influence the vitality of the ovum after fertilization as to lead to its early death and subsequent expulsion. In human beings we tend to focus our attention too exclusively upon the maternal aspects of the problem, and it is quite possible in certain cases of repeated abortions occurring in women with normal genital organs that careful study of the spermatozoa might show that the essential factor is paternal.

The few points mentioned will suffice to indicate that this work is not a treatise for relatively ignorant cow or horse doctors, but rather that it is based upon careful observation and study, and can more than hold its own in competition with many text-books upon human gynecology and obstetrics. Whether all of the conclusions of the author are correct or not I am not in a position to judge, and in many respects it is a matter of indifference, but I can say that the book has interested me immensely, and has demonstrated that the problems of veterinary medicine are as interesting as those of human medicine, and that both can be solved only by the same methods of painstaking and careful scientific work.

J. Whitridge Williams.

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THE SWELLING OF THE ARM AFTER OPERATIONS FOR CANCER OF THE BREAST—*ELEPHANTIASIS CHIRURGICA*—ITS CAUSE AND PREVENTION

By William Stewart Halsted

Nearly 40 years ago, probably in 1882, I had occasion to excise thoroughly the lymphatic glands from both groins of a young man, and there resulted a swelling of the scrotum greater than I have ever seen as a consequence of this operation. Much perturbed, I consulted Dr. Charles E. McBurney who at this time was interested in the management of similar cases, and together, for a year or more, we attempted with inconsiderable success to reduce, chiefly by strapping with adhesive plaster, the distressing dimensions of the swelling. Two and a half years ago this patient, to whose misfortune I had repeatedly referred in my clinics at The Johns Hopkins Hospital and whom I had not seen for about 38 years, came to Baltimore to consult me in regard to another matter. The scrotum, although much reduced in size, was conspicuously large and incommodious. Especially interesting was the account which he gave, in response to careful questioning, of recurrent attacks of redness and increased swelling accompanied by digestive and constitutional disturbances. These attacks, not infrequently ushered in by chills and fever, were milder and less frequent than formerly but still occurred at least once or twice in the course of a year.

Œdema following operative blocking of the lymphatics is most frequently observed after the radical operation for cancer of the breast. For many years I was unable to account for the fact that in some instances a year or two or more after this operation and without return of the disease there would occur, suddenly or perhaps slowly, a swelling, occasionally very great, of the upper extremity. Consultations on the subject with surgeons in this country and abroad were not very helpful, the opinion frequently vouchsafed being that swelling due to obstruction of lymphatics was hard and appeared late, whereas the swelling which followed blocking of the veins was relatively soft and supervened promptly. In the spring of 1914 the Director of one of the greatest clinics in Germany expressed to me the opinion that the typical operations upon the axilla in cases of carcinoma of the breast were unnecessarily radical, and that in order to forestall the excessive swelling of the arm which followed in such a large percentage of the patients he was stripping out the axillary contents in a manner much less thorough. Since adopting this modification he was confident that swollen arms were less frequent in his clinic and that the percentage of recurrence had not been increased thereby.

For many years I have entertained the view that although blocking of the lymphatics and occasionally also of the veins was the underlying factor, infection played a conspicuous part

in the determination of the amount of the swelling and the time of its manifestation. The facts observed in the following case sustain this opinion most convincingly:

A woman (Surg. No. 41383) on whom I had performed at The Johns Hopkins Hospital our radical operation for mammary carcinoma on November 18, 1916, consulted me on May 24, 1920, in regard to a swelling of her arm which had appeared suddenly (within three or four days) three years and three months after the removal of the breast. There had been no swelling whatever of the arm at any time subsequent to the operation prior to an " attack " in February, 1920, in the course of her convalescence from influenza. This attack, as she terms it, was ushered in by nausea, a chill and high fever. The arm promptly began to swell and there appeared " redness in streaks " from the shoulder to the wrist; in a few days the redness was diffuse and the swelling of the arm had become distressingly great; the hand resembled a " boxing glove," and pressure on it with the fingers produced " deep pits." In eight or ten days the redness had vanished and the swelling was rapidly decreasing. During the three months prior to this her second admission to The Johns Hopkins Hospital there had been a less rapid reduction in the size of the affected (left) limb, which about the middle of the arm measured in circumference 9.5 cm. more than the other, and at the middle of the forearm, 3.5 cm. more. No glands were palpable above the clavicle, and there seemed to be no abnormal fullness or resistance below it. In the skin at the outer-upper edge of the grafted area there was recurrence of the carcinoma—two nodules, not ulcerated, one about the size of a filbert, the other smaller than a split pea.

The immediate cause of the swelling was undoubtedly the local infection, for during the three years and three months prior to this there had been nothing to indicate a blocking of the lymph channels.

Eight days after the second admission (June 1, 1920), the patient was operated upon by Dr. Mont Reid, our resident surgeon. The second and a part of the third portion of the axillary vein were found to be completely occluded by the new growth, which was continuous with the larger of the two cancerous nodules in the skin. The entire axillary vein and the recurrent growth were excised in one piece, the disease being given a wide berth. On examination of the specimen it seemed quite clear that the vein had been invaded from without, and had probably been occluded long before the attack of infection. After this operation the swelling rapidly subsided and in the course of two months the arm had almost regained its normal dimensions.

In this case, as in a number of others observed in our clinic, the occlusion of the axillary vein plus the excision of the axillary lymphatics had not been followed by any swelling of the arm.

A few months ago I interviewed a patient (Surg. No. 17659) who each year for the sixteen years following an operation by me at The Johns Hopkins Hospital for cancer of the breast has had one or two and occasionally three or four attacks of redness and swelling of the arm on the operated side. The first symptoms, malaise and nausea, were quickly followed in the severe attacks by a chill and fever and then by slight redness and increased swelling of the arm. The arm of this patient was observed to be swollen a few days after the operation, the swelling being attributed in part to the excision of the axillary lymphatics, but in great measure to a slight infection of the wound along the incision onto the arm.

At that early period, and indeed until perhaps after the introduction of our present operation, about eleven years ago, we believed that, quite independent of infection, swelling of the arm might be expected after the radical operation for mammary cancer.

The initial account of the operation for cancer of the breast which bears my name lies buried in the second volume of the Reports of The Johns Hopkins Hospital (1891) under the title " The Treatment of Wounds with Especial Reference to the Value of the Blood Clot in the Management of Dead Spaces,"[1] and is known only to a few.

Nine years have now elapsed since the publication of my paper on " Developments in the Skin-Grafting Operation for Cancer of the Breast,"[2] in which a modified incision and radical changes in the manner of closing the wound were advocated; but I was not then able to offer an explanation altogether satisfactory to myself of the fact that the swelling of the arm which not infrequently followed our original procedure became almost immediately after the adoption of the modifications described in the latter paper a complication of rare occurrence.

Our operation of to-day is the one which with few exceptions has been employed at The Johns Hopkins Hospital for the past eleven years. Prior to its adoption we had not infrequently observed that extreme abduction of the arm was prevented by a cicatricial, cuticular band in line with the scar of the incision onto the arm. In no case, however, did the patient consider herself sufficiently incommoded by the restriction of movement in this direction to accede to the proposition that the tugging band be divided and the defect covered by skin grafting.

The problem thus thrust upon us was readily solved: the incision down the arm, made shorter and shorter, was soon abandoned altogether, and the skin-edge at the upper margin of the wound was tacked with fine silk sutures to the first intercostal muscle and its fascia in such manner as to raise the axillary fornix to the highest desirable point (*vid.* Fig. 1). Thus the possibility of tug on any part of the skin or scar was eliminated.

No effort was made to approximate the cut edges of the skin at the upper third or perhaps upper half of the denuded area; on the contrary, the flaps, if so they may be called, were pressed away from the wound's centre—the outer flap upwards and outwards, the inner flap upwards and inwards—and stitched to the underlying muscles of the thoracic wall. Thus there was secured for the infraclavicular and axillary regions a superabundance of skin, and entire freedom of arm movements in all directions was assured. Furthermore, by the tacking of the margin of the flap close up to and sometimes even under the subclavian vessels (*vid.* Figs. 1 and 2 and 4 to 12), there could be no dead space below the clavicle such as results in plastic operations from the attempt to approximate the margins of the flaps—the dead space whose arc, formed by clavicle, first intercostal muscle, and first rib, is subtended by the more or less tightly drawn tegmen. The lower half or more of the denuded area was to a limited extent

[1] W. S. Halsted, The Treatment of Wounds with Especial Reference to the Value of the Blood Clot in the Management of Dead Spaces. The Johns Hopkins Hospital Reports, 1891, II, p. 255.

[2] W. S. Halsted, Developments in the Skin-Grafting Operation for Cancer of the Breast. Jour. Amer. Med. Assn., 1913, LX, p. 416.

covered by gently drawing the free margin of the skin towards the centre of the wound and tacking it to the underlying muscles, although the size of the grafted area is of little practical consequence to the patient, and only slightly more time is consumed in covering with the large grafts a greater than a lesser surface.

More was accomplished by this new method than had been anticipated, for not only was the normal range of arm movements secured but there resulted other benefits unforeseen. Swollen arms of dimensions sufficient to distress or annoy the patient were no longer observed, marginal necroses rarely occurred, and the grafts took throughout with few exceptions.

Now it is within the experience of every surgeon, especially of those who employ plastic methods to cover the defect, that frequently marginal necroses of the flaps occur and occasionally sloughings of considerable dimensions, and that it is usually in these cases that the swelling of the arm is most pronounced. Attendant upon the necrosis there is infection, and inflammatory reaction in varying degree, and the greater the reaction the greater in general the swelling of the arm immediate and ultimate.

Moreover, there are, as I have said, cases in which months or even years after the operation on the breast the arm becomes swollen from a cause other than the recurrence of the carcinoma.

Recurrent carcinoma being excluded, may not the later swelling of the arm have the same cause, whatever that may be, as that which appears soon after the operation? What is the cause? What the explanation of the fact that after the radical operation, the world over, for cancer of the breast the arm so frequently swells and sometimes to such degree that amputation has been proposed and indeed made for relief?

Why is it, furthermore, that a modification in our method of closure of the wound, the operative details otherwise being essentially unchanged, should so strikingly affect the result as regards the swelling of the arm?

It is not from observations made in our clinic alone but from the testimony of surgeons and radiologists elsewhere that we have been led to conclude that swelling of the arm follows the plastic operations in greater proportion and in more pronounced form than is seen in the cases treated by skin grafting. And, moreover, in the skin grafting operations, which with us trace back to 1895, conspicuously swollen arms became almost a thing of the past after the method of pressing back the flaps at the upper part of the wound, and stitching their edges to the underlying intercostal muscles was adopted.

Something must therefore have been superimposed upon the mere clearing out of the axilla in the ordinary as well as in the extraordinary plastic operations, and in the operations at The Johns Hopkins Hospital which antedated our modified procedure, to account for the so frequent swelling of the arm.

In addition to the evidence furnished by our clinical experience we have the testimony of the many who have studied the causes of elephantiasis filariosa that blocking of the lymphatic glands and vessels is not of itself sufficient to account for the œdema, at least in its pronounced forms.

Dr. F. L. Reichert, who with the assistance of Dr. C. Y. Bidgood has made for me in the Hunterian laboratory, during the past winter, experiments bearing on this subject, has thus far been unable to produce œdema in the legs of dogs by the mere severing of veins and lymphatics. In these amputations all the tissues of the thigh were severed except the femoral artery and vein, the main nerve trunks and the bone; then the divided parts were carefully reunited by stitching. For 7 or 8 days there would be slight swelling of the leg below the line of suture. On subsidence of this temporary swelling the femoral vein (the only undivided vein) would be ligated. No demonstrable increase in the size of the leg occurred after this ligation. Thus in one week or less the lymphatico-venous circulation was reestablished through the line of suture—through the scar. We have it in mind to make the ligation also of the artery at various periods after the amputation.[3] Attempts are being made by us to determine the relative susceptibility to infection of the two hind legs—of the operated and the normal one. We conjecture that the leg operated upon may prove to be more susceptible to infection than the other and also that an abnormal amount of swelling may follow a successful inoculation of the obstructed leg.

These experiments call to mind an interesting paragraph by W. G. MacCallum on "Inflammation in Tissues Separated from Connection with the Central Nervous System."[4]

> An attempt was made to study the cause of inflammation in tissues separated from connection with the central nervous system as compared with that in normal tissues. The mere section of the nerves going to an organ or limb is insufficient, for nerve fibrils accompany the blood vessels. To overcome this an extremity was amputated completely and replaced by anastomosing the blood vessels and bringing together muscles and skin. Inflammatory irritants applied symmetrically to the intact, and to the amputated limb of the dog resulted in the production of quite the same phenomena of inflammation on both sides. The reddening due to the dilatation of the blood vessels

[3] Since the above was written Dr. Reichert has informed me that he has already carried out the suggestion to ligate the artery as well as the vein about a week after the amputation made in the described manner. Neither gangrene nor swelling followed the ligation of these vessels. Thus, within a week of the time of the amputation of the thigh the arterial and venous circulations had been so well restored through the line of union of the divided tissues as to make possible the ligation of the femoral artery and vein without causing any appreciable swelling or anemia of the limb. It remains to note the effect of ligation of the artery with unoccluded vein, and also to determine the earliest period after the amputation at which these vessels can safely be occluded. It is to me a surprising fact that in so short a time the arterial, venous and perhaps lymphatic circulations can be reestablished through a scar. We have it in mind to inject the vessels of the animals so treated with shadow-casting materials and thus, if possible, study with the X-ray the fine channels through which the new circulation is carried on. Conceivably an amputated limb might be reinstated as is the case with severed fingers, ears and noses. One must at least make the experiment. These experimental observations tend to strengthen my belief that swelling of the arm is unlikely to be caused by the mere excision of the axillary lymphatics. It would seem to be quite certain that another factor is needed, namely, infection.

[4] Proceedings of the Soc. for Exp. Biol. and Med., 1910-11, p. 180.

was perhaps slightly more intense on the amputated side than in the intact limb. Evidently, the control of the central nervous system is not at all necessary for the development of inflammatory changes.

May the swelling of the arm after the radical operation for cancer of the breast be prevented? The study of our cases operated upon by the newer method has brought out the fact that excision of the axillary and supraclavicular glands plus resection of the subclavian and axillary veins is rarely followed by noticeable swelling of the arm. On the other hand, we do not deny that obstruction of these lymphatic and venous channels might conceivably alone, without infection, suffice occasionally to produce a moderate amount of œdema. But it is assuredly impossible to assert in any case that infection has played no part in the causation of the swollen arm.

It is unnecessary to stress further the importance of discarding operative methods which clearly predispose to infection, quite aside from the fact that these methods—the plastic ones—tend to deter the surgeon from sacrificing a sufficient amount of skin or, if perchance enough skin has been excised, to increase the danger of swelling and of restricting the range of movement of the arm. In the plastic operations, the wide dissections of the skin extending sometimes to the spinal column, over and under the opposite breast and down to the iliac crest, may conceivably sever lymphatics which might aid in the restoration of the lymphatic circulation. The performer of plastic operations presumably asks himself whether skin enough to make possible the use of his favorite method can be preserved; whereas the advocate of skin grafting who understands its possibilities may sacrifice, without concern about the closure of the wound, as much skin as seems to him desirable. It is unfortunate that surgeons should permit themselves to be placed in the predicament of choosing either to incur greater danger of recurrence or to imperil the success of a plastic method.

During the past year we have been making a careful search for cases of swollen arm following operation at The Johns Hopkins Hospital for cancer of the breast and, although at this writing we are not prepared to submit figures, may state that the records support our view that infection is very frequently, if not indeed usually, the overlying cause of the swelling of arms whose main lymphatic channels have been more or less blocked by operation. The infection may quite conceivably be so mild in degree as to escape the observation even of those intently on the lookout for it.

If the view expressed in this paper as to the cause of the swelling of the arm following operations upon the axilla should prove to be correct the term *surgical elephantiasis (elephantiasis chirurgica)* might be an appropriate one.

The most common cause of the late postoperative swelling is, of course, the recurrence of the disease, a recurrence which blocks new channels. But swelling in its most aggravated form is seen with the recurrences accompanied by inflammation—it may be only the reactive inflammation incident to the rapidly growing neoplasm. Now the question naturally presents itself as to whether the reactive inflammation in some of these recurrent cases may not be partly of bacterial origin—an inflammation superimposed upon the tissues engorged from lymphatic and frequently also from venous obstruction. It would seem to me to have been determined from clinical observation that lymphatic obstruction predisposes to streptococcal inflammation, but it remains for us to confirm this hypothesis by experimental demonstration on animals. We have been impressed with the fact that lymphangiomata seem predisposed to infection, and to recurrent attacks of inflammation.

The clinical and experimental evidence bearing on the part played by streptococcal infection in the production of elephantiasis and elephantoid conditions has been admirably summed up by Matas:[5]

By elephantiasis we mean a progressive histo-pathologic state or condition which is characterized by a chronic inflammatory fibromatosis or hypertrophy of the hypodermal and dermal connective tissue which is preceded by and associated with lymphatic and venous stasis, and may be caused by any obstruction or mechanical interference with the return flow of the lymphatic and venous currents in the affected parts. In order to bring about the hypertrophy of the connective tissue, which is the distinctive feature of the true elephantiasic state, the mechanical impediment to the lymphatic and venous drainage of the part is not sufficient, because a simple mechanical obstacle, while causing a regional or localized dropsy or lymphedema, will not bring about the characteristic fibromatosis and other histologic changes which are peculiar to elephantiasis. As Unna, Darier and many others have well shown, a simple mechanical edema is incapable of exciting a proliferation of the collagenous connective tissue. We know by clinical observation that edema may exist many years in the extremities and other parts without causing any fibromatosis or hyperplasia of the connective tissue of the parts. Something more than lymph stasis is required, and that something is infection with pathogenic organisms, and especially those of the streptococcal type which find a favorable soil for development in the stagnant lymph stream. The histopathological elements which are essential to complete the picture of elephantiasis are: (1) a mechanical obstruction or blockade of the veins and lymphatics of the region, usually an obliterative thrombo-phlebitis or lymphangitis or adenitis; (2) hyperplasia of the collagenous connective tissue of the hypoderm; (3) gradual disappearance of the elastic fibres of the skin; (4) the existence of a coagulable dropsy or hard lymph edema; and, (5) a chronic reticular lymphangitis caused by secondary and repeated invasion of pathogenic micro-organisms of the streptococcal type.

If we adopt this conception of the histologic process which underlies the pathology of elephantiasis as it is recognized in its endemic tropical types, as well as in the sporadic cases, which may occur in all climates, we can readily appreciate that the histologic process is of a generic character, though it may be initiated by many specific causes—the underlying histologic background remaining, however, always the same. In this manner, we can easily reconcile the many conflicting views relative to the pathology of elephantiasis. The long established duality of classification of the disease into the classic *Elephantiasis tropicum,* which is usually attributed to the presence of *Filaria nocturna,* or the strictly parasitic type of the disease, and the *Elephantiasis nostras streptogenes,* which prevails in all climates, no longer suggests distinct and specific types of the disease. These are histologically alike as morbid processes, and only differ in the primary cause of the lymphatic and venous obstruction which initiates the process. Therefore, while nosologists classify elephantiasis into the

[5] The Surgical Treatment of Elephantiasis and Elephantoid States Dependent Upon Chronic Obstruction of the Lymphatic and Venous Channels. With Case Reports by the Author and Hermann B. Gessner, M. D., School of Medicine, Tulane University of Louisiana, New Orleans. Amer. Jour. of Trop. Dis. and Prev. Med., 1913, I, 60-85.

Fig. 1.—The redundant axillary skin is drawn up by the fingers. The skin has been stitched to the underlying muscles at the margin of the area to be grafted, the apex of which corresponds to the lower border of the first rib. Reproduced with permission of the *Journal of the American Medical Association.*

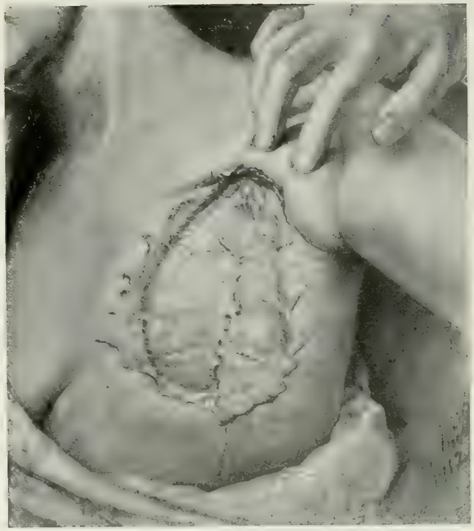

Fig. 2.—The defect has been covered with Ollier-Thiersch grafts. The redundant axillary skin retracted by the fingers, as in the preceding illustration. Reproduced with permission of the *Journal of the American Medical Association.*

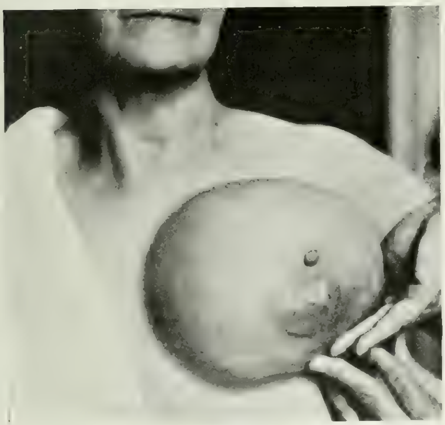

Fig. 3.—S. N. 29519. Operation by Dr. Halsted March 30, 1912. Reproduced with permission of the *Journal of the American Medical Association.*

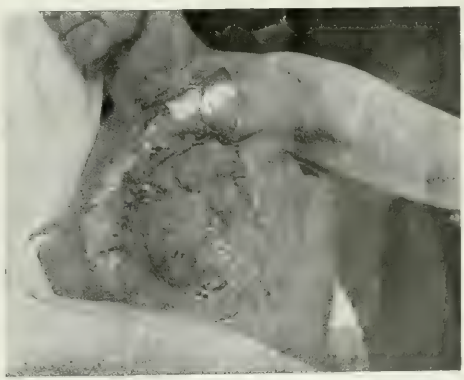

Fig. 4.—S. N. 29519. Three days after operation. The denuded area covered by skin grafts. Silver-foil (high lights) on the skin along the inner and upper margins of the wound. Reproduced with permission of the *Journal of the American Medical Association.*

Fig. 5.—S. N. 51869. Operation by Dr. Reid September 3, 1920. Huge fungating carcinoma; skin metastases; subclavicular, axillary, and regional œdema; swollen arm, forearm and hand. Photograph taken nine months after operation. The swelling of the arm was not increased by the operation. Note the great size of the grafted area and the redundant skin in the axilla.

Fig. 6.—S. N. 49379. Operation by Dr. Reid September 19, 1919. Photograph taken April 1, 1921.

Fig. 7.—S. N. 49808. Operation by Dr. Heuer November 21, 1919. Photograph taken January 18, 1921.

Fig. 8.—S. N. 37632. Operation by Dr. Dandy August 10, 1915. Photograph taken Feb. 27, 1920.

FIG. 9.—S. N. 46656. Operation by Dr. Reid September 27, 1918. Photograph taken February 27, 1920.

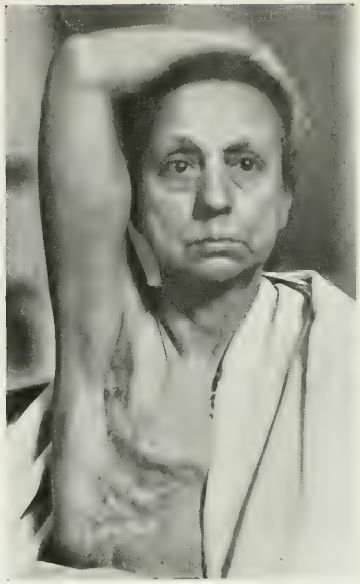

FIG. 10. S. N. 40261. Operation by Dr. Dandy July 12, 1916. Photograph taken March 8, 1918. A graft placed almost at the very top of the axillary fornix.

FIG. 11.—S. N. 42198. Operation by Dr. Heuer February 19, 1917. Photograph taken March 8, 1918.

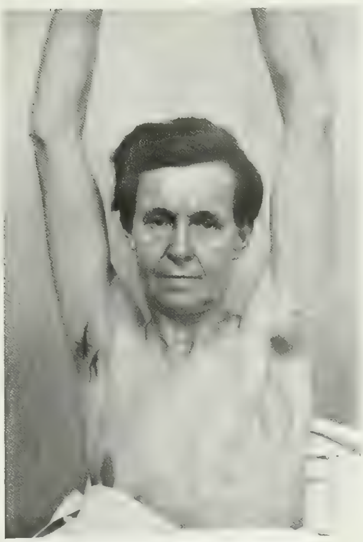

FIG. 12.—S. N. 45095. Operation by Dr. Follis March 1, 1918. Photograph taken February 27, 1920.

previously mentioned groups, it is evident that the elephantiasic process is always one and the same; always maintaining similarity of histologic type regardless of the multitude of causes that may bring it into existence. The one etiologic factor which seems to be inseparable and essential to its pathogeny is infection—frequently repeated—which brings about permanent alterations in the vasculolymphatic apparatus of the skin and its underlying connective tissue. The reason why elephantiasis, as a clinical entity, is so much more frequent in the tropics than elsewhere, is because the conditions which predispose to and favor cutaneous infection as well as lymphatic obstruction, are so much more frequent there than in colder climates; greater exposure of skin areas, especially the lower extremities, to traumatisms of all sorts; greater activity and, consequently, greater irritability of the skin; universal presence of suctorial insects and gross parasites; and greater frequency of eruptive diseases, etc., all of which tend to open wide the portals of infection. It is now pretty well admitted that filariasis is only one of the causes that initiate the elephantiasic process even in the tropics. Certainly, the filariæ are not so frequent a cause of the disease as was formerly believed. The incidence of filariasis is not coincident with that of elephantiasis. For instance, Creighton Wellman (Journal of Trop. Med., 1908, p. 118), while stationed as health officer at the Portuguese Colony of Angola, in West Africa, had many opportunities for investigating the relationship between filarial disease and elephantiasis and in a careful study of fifty cases he never found any evidence of embryo filariæ in the blood, or for that matter, in the blood of five hundred individuals in that colony, whom he examined for evidence of filarial disease. Le Dantec had a similar experience in French Guiana, where elephantiasis is common and filariasis very rare. Prout, in Sierra Leone (1908), observed a great many cases of filariasis in that colony, but met with a similar experience in the Congo, where filariasis is almost a universal disease. Sir Patrick Manson, who immortalized himself by his researches into the mode of propagation of filarial disease through the agency of the mosquito, and was the first to establish the relations of filariasis to elephantiasis, has modified his earlier views on the relationship of filiarial parasitism to elephantiasis. In his work on Tropical Diseases (1907) he now teaches that the mechanical obstruction of the lymphatics caused by *Filaria bancrofti* is not alone sufficient to cause elephantiasis, and that a secondary microbial infection of the obstructed area is necessary to cause the tissue changes of elephantiasis. This same observer writes:

"Lymphatic stasis, by itself, does not cause elephantiasis. The obstruction may cause lymph edema, but not a true hypertrophy of the edematous tissues. If an inflammation (infection) is acquired in a closed area of lymphatic congestion, an event which may result as a consequence of the slightest traumatism, elephantiasis will then develop."

According to Low, if a lymphatic obstruction caused by adult filariæ is followed by an attack of erysipelas, the filarial embryos are killed and their disorganization is a cause of the lymphangitis which culminates in elephantiasis. Clinical observation also abundantly shows that if a lymph scrotum develops as a consequence of filarial obstruction, it will remain a plain lymph scrotum for an indefinite period of time, and will only become an elephantiasic scrotum when repeated attacks of erysipelatous infection follow in the wake of the mechanical stasis and in this way starts the fibromatous process which is the histologic essential of elephantiasis. The burden of this discussion is merely to show that even in tropical climates where filariasis prevails endemically, elephantiasis is a diseased state not subject to one cause, but to many, and, in fact, to any agency that may obstruct the lymph stream provided dermal infection follows in the wake of lymph stasis. We have now come to realize that whatever the cause of the lymph edema may be, the element of infection is the one essential and determining factor in the production of true elephantiasis. In fact, if we accept the views of many writers, such as Le Dantec, Sabouraud and Unna, the progressive fibromatosis which we recognize as *Elephantiasis nostras,* may occur independently of any stagnatory state of the lymphatic or venous circulation, and solely as a result of repeated attacks of a streptococcal infection, which has been regarded by many as identical with the erysipelas coccus of Fehleisen. Moreover, the streptococcal infection of elephantiasis presents all the characteristics of the classical cutaneous erysipelas, with the exception that in elephantiasis it is usually limited to one particular region in the lower extremity, the eruption rarely extending beyond the groin. It is rarely ambulatory or migratory as is the case with the well-defined type of erysipelas.

This association of a streptococcal infection of the erysipelatous type is, we repeat, almost an inseparable and constant feature of elephantiasis, no matter what the original predisposing cause may be. It is also this association of the erysipelatous attacks, with the progressive hyperplasia of the dermal and hypodermal connective tissue of the affected region, that distinguishes true elephantiasis from the elephantoid states. Sabouraud, who has given much attention to the bacteriology of elephantiasis, Unna, Le Dantec, Bockhart, and others, agree that the micro-organism is a distinct *Streptococcus* which cannot be morphologically distinguished from the Fehleisen coccus of erysipelas. Le Dantec describes in addition a special coccus which he designates as the lymphococcus; Renon found the pneumococcus. But there is no question that from the clinical point of view, the recurrent streptococcal infection which is peculiar to elephantiasis is similar to that of erysipelas. In the many cases that have come under our observation, the history of repeated attacks of erysipelas has been invariably connected by the patient with the origin and evolution of the disease. Sir Joseph Fayrer applied the term elephantiasic fever to the febrile manifestations which accompany the appearance of the local erysipelatous rash. The fever is usually ushered in by chill or rigors, and the temperature rises to 103° or 105° F. and is followed by remissions and profuse sweats. The fever usually lasts from two to eight days and defervesces with the subsidence of the erysipelas, which rarely extends beyond the root of the limb. During the attack, the skin of the affected region becomes red, painful, swollen, and looks exactly like the erysipelas rash; the lymph nodes are enlarged and the redness diffuses itself with great rapidity over the entire surface of the limb. After the subsidence of the attack, the skin remains swollen, giving the impression of a soft, doughy edema. The soft consistency gives place to a more permanent hardness, the skin of the affected part never returning to normal. The underlying soft parts unite with the skin, which increases very much in thickness, forming a firm, immovable mass of tissue which continues to grow until it finally assumes a monstrous size (Elephant's foot, Barbadoes leg, etc.) which is characteristic of the disease.

The development of elephantiasis by inoculation of pure erysipelous cultures was experimentally tested by Bockhart, Sabouraud, Le Dantec and others. In accounting for the repeated or recurrent attacks of this streptococcal infection, Unna (Histopathology of the Skin, Walker's translation, p. 493) states:

"It is in the highest degree probable that the true sporadic cases of elephantiasis develop from incompletely healed erysipelas—that is, those which leave behind disturbances in the circulation. The streptococci remain latent in the tissues and in this way excite the chronic proliferative tissue changes which we subsequently recognize as elephantiasis. At first the attacks of erysipelas occur at long intervals, perhaps twice or three times a year, then once a month, rarely oftener. Each time the limb grows larger until in the course of time a distinct elephantiasic state is established. Natural immunity does not appear to be easily acquired; and once the erysipelatous habit, so-called, is acquired, it usually becomes a permanent feature of the disease, though in some cases the attacks become milder and disappear altogether, but not until the elephantiasic state is fully established."

STUDIES ON BLOOD

THE VITALLY STAINABLE GRANULES AS A SPECIFIC CRITERION FOR ERYTHROBLASTS AND THE DIFFERENTIATION OF THE THREE STRAINS OF THE WHITE BLOOD-CELLS AS SEEN IN THE LIVING CHICK'S YOLK-SAC

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In 1920 the writer[37] published an account of a study of the origin of the vascular sytem as it can be made out by watching the living chick blastoderm of the second day of incubation. The method of the origin of vessels can be made out in such specimens, in the area pellucida of the yolk-sac, in stages which range from the time just before the first somite through the stage of about twenty somites.

In the area pellucida there are three well-known layers, the ectoderm, the double layer of the mesoderm lining the extra-embryonal cœlom, and the endoderm. The blastoderms are mounted in a hanging drop preparation, in Locke-Lewis solution, with the endoderm against the coverslip. The area pellucida is so thin that the endoderm, the vascular zone between the endoderm and mesoderm, and the mesoderm can all be analyzed with an oil immersion lens. The technique was described in 1920.

Blood-vessels begin by the differentiation of a new type of cell from mesoderm. This cell moves out of the mesoderm, develops a dense, basophilic, azurophilic cytoplasm and becomes physically more refractive than mesoderm. As soon as this cell divides, it shows one of its essential characteristics, namely, the tendency of the cells to stay together to form syncitial masses. These masses of cells put out sprouts by which they join similar masses to make a plexus. Both, while such solid clumps are still isolated, and after the plexus is formed, the cells become transformed into vessels by a liquefaction of the central part of the mass to make blood-plasma, while the periphery differentiates into endothelium.

There is a progressive differentiation of angioblasts, beginning in the periphery of the area vasculosa at the stage of two somites; the cells gradually appearing nearer and nearer the embryo, until, at the stage of five or six somites, angioblasts differentiate in the axial line of the embryo as forerunners of the endothelium of the heart and aorta. The heart, aorta and main vessels of the embryo differentiate *in situ* from angioblasts and increase by the addition of newly differentiated cells as well as by the cell-division and the sprouting of their endothelial walls. The amount of the differentiation of new cells grows less but at what stage it ceases is not known. Thus there is established the fundamental morphology of the vascular system.

Blood-vessels arise by the development of a new type of cell, the vasoformative cell of Ranvier, or the angioblast of His. This cell produces the first fluid of the blood; thus, endothelium is primary and blood-plasma secondary. Since there is a tissue fluid before angioblasts arise, endothelium is from the start a membrane between two different fluids, tissue-fluid and plasma. Moreover, the process of liquefaction is intra-cellular, that is, it can be seen in chains of single angioblasts which become vessels and it can also be seen to take place in the sprouts which are processes of cells, hence the lumen of vessels is embryologically intracellular and thus not a tissue-space.

In the living chick of the second day, it can be seen that both angioblasts and the endothelial cells give rise to red blood-cells. Erythroblasts begin in the chick in the vessels of the outer margin of the area opaqua in the stages of from 7 to 11 somites. In the area pellucida, where they can be seen in the living specimen, angioblasts differentiate during the stages of from 5 to 11 somites, while the vessels form and erythroblasts begin during the stage of from 11 to 14 somites. The heart begins to beat at the stage of 10 somites and the circulation starts when the chick has 16 to 17 somites.

During the past year, I have been continuing these studies on the living chick, and have found that it is possible to mount the entire blastoderm on a large cover-slip, one measuring 42 by 50 mm., throughout the third and fourth days of incubation. The cover-slips must be entirely free from grease or the membrane will not flatten out on the glass. From the fifth day on, the chick is too heavy to mount in the hanging-drop form, but if the specimen be transferred to a dish of Locke-Lewis solution, the amnion can be opened, the allantois pushed aside and the yolk-sac cut off close to the embryo and then spread out and mounted. The circulation, of course, stops but the membrane can be mounted and kept alive certainly for from three to five hours. So far, I have studied these living membranes only through the first seven days of incubation. It is an advantage to mount the chick with the membranes because the preparations are all fixed after the vital studies have been made and many of them are stained and mounted *in toto*. If the embryo has been left attached, it can be cut off in the alcohol and then the entire ectoderm can be dissected off from the area vasculosa. The specimen is thus made thinner and much easier to analyze. When the embryo has been cut away at the start, the ectoderm clings too closely to the specimen to be taken off and makes one more layer of stained cells in the final specimen.

I have found it a great advantage to study the embryos with a vital dye and add 1 to 3 drops of a 1 per cent aqueous solution of neutral red to 10 c. c. of the Locke-Lewis solution. making a dilution of possibly 1 to 10,000 of the dye, or less. This may be termed the physiological dilution of the dye.

After the specimens have been studied, they are fixed by floating the cover-slip, embryo down, on Bouin's picro-formol and then keeping them in 70 per cent alcohol until all the picric acid is removed. They are then stained in hæmatoxylin and counterstained in eosin with a little orange G. This fixation is excellent for the granulocytes, but quite worthless for the erythrocytes after the primitive stage. After fixation in Bouin's solution the young erythrocytes show only a wide-meshed reticulation having no relation whatever to any of the substances that can be made out in the living cell. No fixation of the blastoderms is adequate to follow the changes in the red cells, but they can be identified best after fixation in the vapor of formalin if not applied too long. I use it for from ten minutes to half an hour. In this the hemoglobin is well preserved but not the basophilic cytoplasm and indeed the earliest traces of hemoglobin which can be seen in the living cell by a distinct yellow color cannot be detected in the fixed specimens. Helly's fluid, which preserves the basophilic substance better, cannot be used, because the blastoderms float off from the cover-slip almost immediately and wrinkle so that they can never be studied as a section with an oil immersion lens.

In connection with the study of these blastoderms, a drop of blood is drawn from the vessels when the egg is first opened and used for a film. These films of blood are studied vitally by Pappenheim's method. They are made as follows: A clean glass rod is dipped into a dye and drawn across a perfectly clean glass slide. The two dyes which I have used are neutral red and brilliant cresyl blue, made up either in a 1 per cent aqueous solution or in a saturated alcoholic solution. The even film of the stain dries quickly and its strength is estimated by the color. The film must not be dense enough to stain any nuclei. A drop of blood is then drawn out with a fine glass cannula, placed on a cover-slip which is then inverted on the film of stain, ringed at once with salvoline and placed in a warm box. The vital stains develop slowly, on an average, in ten minutes, and if permanent preparations are wanted, the specimen is watched under an oil immersion lens until the staining is best, then the cover is drawn off from the slide and the film of blood counterstained with one of the blood stains. I have used Wright's eosin-methylene blue. In blood from chicks on the second and third days of incubation the amount of the specific substance of the red cells, that is, the megaloblasts, stainable in the vital dye is so massive, that it is necessary to differentiate the specimens after staining by Wright's method. This can be done in absolute alcohol and the decolorizing stopped with xylol. The white cells are so very unevenly distributed in the vessels of the early blastoderms that no film of blood represents adequately the amount of differentiation for the stage. Hence, each stage must be studied by both methods, by a survey of the total area pellucida and by drops of blood with specific stains.

In these studies, it can be seen that there are three different strains of blood-cells, first, those that arise from endothelium, which include both the red cells and the monocyte strain of the white cells; secondly, the granulocytes, and thirdly, the lymphocytes. The red cells begin to differentiate on the second day of incubation. On the third day the endothelium gives rise to the monocytes, that is, to the large mononuclears and the transitional forms of Ehrlich. In two different specimens I have seen an occasional monocyte on the second day, but the process becomes active only on the third day. The group of the monocytes of the blood is especially well illustrated by Pappenheim on his Plate 1,[33] as the third row of cells, and as the fourth and fifth rows on Ferrata's Plate 12.[12]

At the same time that the endothelium gives rise to the monocytes, namely, beginning on the third day, it gives rise to a much more numerous extravascular group of cells, identical with the monocytes, which are the clasmatocytes of the connective tissues. On the third day also the granulocytes begin to differentiate as a new type of cell from the mesoderm. These cells develop a specific type of granulation and wander into the blood-vessels. The third strain is the lymphocytes. These I have never seen differentiating in the wall of the yolk-sac; they begin to appear in the blood stream on the fourth day but do not become marked until the fifth and sixth days. It may be that they arise only within the embryo itself.

ERYTHROBLASTS

In these studies it has been possible to establish a criterion for a primitive red cell. As was discovered by Pappenheim,[31] the primitive red cell has a basophilic, azurophilic cytoplasm so finely granular as to appear like ground glass. Fixed to show this basophilic cytoplasm, the cell looks like a single angioblast. In the living cell a droplet of yolk is occasionally to be seen. If, however, one stains the cell supravitally with either neutral red or with brilliant cresyl blue, there appears a very massive granulation which at first completely fills the cell. This granulation is completely soluble in alcohol and in all of the usual fixatives; it disappears also in the vapor of formalin. If, however, it be stained with neutral red or with brilliant cresyl blue, it becomes insoluble in methyl alcohol and hence can be seen in films stained with Wright's eosin-methylene blue. For these double stains brilliant cresyl blue is slightly better. This special granulation is then very easy to bring out in films of blood. It is not so easy to stain in the total blastoderm, because the dilution which stains the neutral red granules of endothelium, the yolk and all of the stainable substances of the clasmatocytes, that is, the dilution of 1 to 10,000, is too weak to stain the granulation of the reds, but it does, nevertheless, make it just visible. The primitive red cell in the living blastoderm can be stained, however, by injecting the dye directly into the blood-stream; or if a drop of 1 per cent solution of neutral red is put on the blastoderm for a few seconds and then washed off with clear Locke-Lewis solution, the red cells show the stain well.

The question must come up as to whether the stainable substance actually exists in the living cell in some state from which it is precipitated by the dye, just as Mott has shown that the Nissl substance, in its stainable form, develops only as the nerve cell dies. Indeed, when Israel and Pappenheim[17] first described the vital staining of substances in erythrocytes, they

saw that the staining commenced only as the cell began to die. This may be true. The criterion I have used for the actual death of the cell is whether the nucleus stains or not, and this specific substance does stain in dilutions which do not stain the chromatin of the nucleus at all. The exact dilution necessary to stain it must be worked out.

For the permanent films of blood on the second and third days, the granulation is so dense that, after counterstaining with Wright's stain, the cells must be differentiated in absolute alcohol, the decoloration being stopped with xylol.

On the second and third days, the red cells being megaloblasts, both the granulation and the basophilic cytoplasm completely fill the cell. On the fourth day a narrow rim of clear cytoplasm appears in many of the cells making erythroblasts, showing hemoglobin around the edge free from granules, while the granules make a very dense rosette or wreath around the nucleus. These rosettes are very characteristic and in stained preparations they greatly obscure the nucleus. By the fourth day the red cells have no longer the somewhat uniformly round shape of the earlier stage and there is no longer a comparative uniformity in size, but rather there are many much larger forms together with many small irregular or oval cells. This period of great variation in size is a stage of active division of the cells as well as of growth of individual cells. In the small oval cells, the rosettes are oval and in division the rosettes divide, so that each daughter nucleus is surrounded by a wreath of granules before the cells separate.

On the fifth day a few of the red cells begin to show a diminution of the granulation, some of the cells grow much larger and the granules and rods begin to spread out into the cytoplasm, the cells showing the polychromasia and the reticulation which is known to be characteristic of the so-called reticular forms occuring in anæmias in human blood. By the seventh day there are large numbers of the cells in the reticular stage, while there are still many of the primitive cells and the wreath forms. Gradually the amount of the basophilic cytoplasm and of the vitally stainable granulation decreases while the hemoglobin increases. Finally, the basophilic cytoplasm disappears, but a small amount of the specific granulation remains in each erythrocyte. At the time of hatching and for three days afterward, all of the red cells in the circulating blood show from one to eight or ten vitally stainable granules. I have not carried the studies farther nor studied the stages between the seventh day of incubation and the time of hatching. It is clear, however, that one can work out the types of cells that are characteristic for each stage of the developing chick. Of course, at any stage there are some cells characteristic of preceding stages. On the second day only the primitive stage, the megaloblast, is present, and then, with a given increment of time, the different stages in the development of this specific granulation are added. When such a study has been made for a mammalian form and especially for the human embryos, as is now feasible, we shall be in a position to estimate just how primitive are the cells that appear in the circulation in anæmias.

The first account of the staining of the specific granulation of the red cells, which I have been able to find is in a paper by Israel and Pappenheim[17] in 1896, in which they say that if a few dry grains of neutral red are placed on a slide and used for a film of fresh blood, there will appear a granulation in some of the red cells just as the cell begins to die. In 1901, Bettmann[2] described the use of vital neutral red in the staining of red cells in pathological conditions, but did not discriminate between a staining of the nucleus and the specific granulation around it. Three years later, Rosin and Bigergeil[35-36] described the methods of vital staining and the dyes to be used, but it was not until 1907 that we have a clear account of the vitally stainable granulation of the red cells. In 1907 there appeared three papers in the Folia Hæmatologica, by Caesaris-Demel,[4] by Pappenheim[34] and by Ferrata,[13] in which the vitally stained granules are described and illustrated. Caesaris-Demel shows the stage of the wreath around the nucleus, not, however, in as primitive a stage as on the fourth day of incubation in the chick. He shows also the reticular stages and the final stage of a few granules. He distinguished between the deeply staining granules and the more faintly staining filaments.

The primitive basophilic cell, which is the first red blood-cell, was first differentiated by Pappenheim and called the megaloblast. It has a basophilic cytoplasm and a large nucleus, poor in chromatin, and a conspicuous nucleolus. It becomes an erythroblast and all the stages of the development of this cell, as far as concerns the decreasing of its basophilic cytoplasm, the increasing of its content of hemoglobin and the changes of its nucleus, have been worked out with the eosin-azur technique in final perfection by Pappenheim,[31-34] Ferrata,[12] Danchakoff,[5,6] Maximow[22-24] and Weidenreich.[39-41] The development of this cell with reference to its specific granulation is now necessary to complete its life history.

In the chick, it has been shown that all of the primitive blood-cells on the second day of incubation are megaloblasts which become erythroblasts as soon as a trace of hemoglobin can be made out. These cells are derived from angioblasts and from endothelium. As far as the specific granulation is concerned, the first stage, on the second and third days of incubation, has the granulation throughout the cell. From the fourth to the sixth day, there are the rosette or wreath forms in which the granulation is around the nucleus. On the seventh day most of the cells show the reticular form of the granulation. All of these stages show diffuse basophilia. With further studies it will be possible to tell just when basophilia and the reticular forms disappear in the majority of the red cells. At the time of hatching all of the cells in the circulation have acidophilic, hemoglobin-bearing cytoplasm with a few vitally stainable granules. It is, of course, clear that at any stage in development, while the majority of the cells are in a specific phase, a few of the earlier types may be found. In normal human blood about one per cent or less of the erythrocytes show a few vitally stainable granules.

The question which must come up first in connection with this granulation is its relation to the so-called basophilic punctation, and both Pappenheim and Ferrata agree that these two substances are entirely different. The basophilic punctation stains in azur after fixation and Ferrata [12] thinks that it is an abnormal clumping "conglobation" of the azurophilic cytoplasm, and he shows the gradual production of the punctate forms in red cells after experimental lead poisoning on his Plate VIII. It is thus easy to see why basophilic punctation does not occur in embryonic blood, whereas the vitally stainable granulation is specifically characteristic of development.

Another point in which this specific granulation may prove of interest is that it offers a chance to study the development of hemoglobin in the cell by testing the granulation for the presence or the absence of iron. Both the azurophilic cytoplasm and the granulation disappear as hemoglobin develops, but the granulation alone is characteristic of the red cell as distinct from all other blood cells.

In the developing blood there are always a few cells containing the Howell-Jolly bodies. These are fragments of nuclei staining just like chromatin, which were discovered by Howell,[16] in 1890, in a study of the blood of the cat after hemorrhage. Of course the corpuscles of the chick are all nucleated, so that the question of the extrusion of nuclei does not come up, but an occasional cell, from the very beginning of the formation of blood on the second day, shows a fragmented nucleus. I interpret such cells as dead. They are to be found in the early blood islands before the cells become free and are very interesting as showing that cell death occurs in the early stages of marked cell-division and growth.

Origin of Monocytes and Clasmatocytes from Endothelium

The separation of the clasmatocyte as a distinct type of cell of the connective tissues is due to Maximow.[20] He showed that by introducing two sterile cover-slips under the skin in rabbits, one could separate three types of cells by the speed with which they passed between the covers, leucocytes appearing first; a special cell, the clasmatocyte, wandered in during the first nineteen hours and the fibroblast in from two to four days. Then he showed that the clasmatocyte was specifically sensitive to neutral red,[21] while Bouffard,[8] Goldman [14, 15] Evans,[7–11] Schulemann [7–9] and a large group of workers have demonstrated that it is the cell of the connective tissues most specifically differentiated to phagocytize and store particulate matter. The specific reaction of this cell to vital, neutral red is that the dye stains certain granules of the cell and certain large fluid spheres which are called vacuoles, the so-called "neutral red granules and vacuoles" of Lewis and Lewis.[18] These vacuoles are organs into which the cell passes phagocytized particulate matter. In the vacuoles the fine particles which the cell has taken up become clumped and, as Evans and Scott [11] have shown, may even be re-crystallized.

Aschoff and Kiyono [1] then showed that an identical reaction to a vital dye could be obtained by certain cells of the blood, namely the group Naegeli [30] has called the monocytes, which are the large mononuclear and transitional forms of the Ehrlich school. Thus they distinguished and related histiocytes of the blood and histiocytes of the connective tissues. Moreover, they regarded the histiocytes of the blood as of endothelial origin.

Pappenheim and Ferrata have illustrated the separation of the monocytes, the leucocytes, and the lymphocytes on purely morphological grounds, believing that there is a common stem-cell, a hypothetical hæmatoblast for them all. Aschoff and Kiyono separate the monocytes, calling them histiocytes of the blood, on a physiological basis, and I think that I can demonstrate on a fundamental embryological basis that the monocyte and the clasmatocyte are identical cells, derived from endothelium, thereby making one of the three great groups of connective-tissue cells that contribute to the blood.

If a blastoderm of the third day of incubation be stained in vital neutral red, the endothelium stands out with numerous granules staining in the dye which are both around the nuclei and scattered in the thin periphery of the cytoplasm. The endothelium of the capillaries and the veins often becomes reduplicated. Endothelium is more refractive than mesoderm, and this property, as well as the granules stainable with neutral red, characterizes both of these layers of endothelium. One of the cells of the inner row can then be seen to enlarge, protrude into the lumen and develop the vacuoles which are characteristic of clasmatocytes. The periphery of the cell then puts out a film of cytoplasm in which there is a central process more refractive than the rest, simulating a flagellum, and these films are in constant motion. In fact the eye is attracted to these cells both by the stained vacuoles and by the motion of the peripheral films of cytoplasm. Such a cell then gradually becomes free. The characteristic motion of the peripheral films continues, keeping the surrounding fluid moving, though the cell itself shows very little locomotion. In the study of the origin of the red blood-cells on the second day of incubation (Sabin)[37] it was noted that the erythroblasts formed great clumps of cells attached to the inner surface of a complete endothelium. The monocytes, on the other hand, differentiate and drop off as single cells, leaving the original endothelial cell from which they came as the wall of the vessel.

An endothelial cell may become phagocytic while it is yet in place, for I have seen them with red cells engulfed just as Maximow [23] shows for a mammal in his Fig. 4 on Plate XVIII. This means that an endothelial cell which is actually a part of the wall of a vessel, not one of the reduplicated forms already on the road toward becoming free, may be phagocytic. That is to say, endothelium is itself phagocytic as well as having the power to give off free cells which are phagocytic. In the same figure quoted above, Maximow shows three free monocytes, very characteristic, labeled Edph. He recognized them as desquamated endothelium but did not identify them as monocytes. In fact all of the early stages of the develop-

ment of blood are beautifully illustrated by the two plates of Maximow in this same article.

While these few cells are getting free in the lumen of the vessel to make the monocytes of the blood, the outer row of the reduplicated endothelium divides rapidly in irregular patches, making the outlines of the vessels, both capillaries and veins, have an exceedingly irregular contour, very different from the smooth contour of the earlier capillaries and from the wall of the omphalomesenteric arteries which now have a single layer of smooth muscle. The clumps of cells along the outer wall of the vessels develop the vacuoles characteristic of clasmatocytes and become free as clasmatocytes. Many hundreds of the extra-vascular cells are formed from the endothelium to one intra-vascular. The extra-vascular forms tend to be larger and have larger vacuoles, but I have seen one of the large cells wander into a vessel. The original endothelium has granules that stain in neutral red; it may also have vacuoles. The free cells all have both vacuoles and granules and a differentiation of the periphery of the cell into motile films. Studied with vital neutral red, the monocytes and the clasmatocytes are conspicuous because they are stained.

Thus, in the early chick, endothelium gives rise to two groups of cells, the megaloblasts which develop hemoglobin and become erythroblasts and a strain of cells termed histiocytes by Aschoff. The extra-vascular histiocytes have been termed clasmatocytes, the intra-vascular ones monocytes. They are identical and are specifically differentiated along the line of phagocytosis. They take up particulate matter and debris in solid form which they segregate and store in certain preformed vacuoles filled with fluid. They do not store this insoluble material permanently because it is gradually returned to the circulation and excreted by the kidney. So they represent a mechanism for taking care of foreign matter in excess of the amount that the body can excrete at the time. In the blastoderms from the third to the seventh day there is comparatively little differentiation of new angioblasts in the area pellucida. In fact, in about fifty specimens, I have found only three masses of solid angioblasts. The hollow isolated vesicles made from these solid masses are, however, more numerous, indicating that this stage lasts longer than the solid stage. In one specimen of the third day of incubation there was a long mass of solid angioblasts which started to liquefy to form a vessel, and while the center of the mass was liquefying to form a vessel, two cells wandered off from the periphery as clasmatocytes. Thus angioblasts can also give rise to clasmatocytes.

If one takes the group of monocytes as they are shown in the third row of Pappenheim's Plate I,[33] or the fourth and fifth rows of Ferrata's Plate XII,[12] it will be seen that they include all of the large mononuclear forms and the transitionals of the circulating blood. Both of these types can be seen early in the chick coming from endothelium; an endothelial derivative which is larger and less vacuolated is the mononuclear cell, a smaller and more vacuolated type, the transitional. The transitional is thus shown to be a finished type of cell like the cell of the adult form, for which the term transitional is therefore a misnomer. The large mononuclear type always has an excentric nucleus; it is distinguished most easily in the films of blood, stained with brilliant cresyl blue and counterstained with eosin-azur. It lacks the specific granulation of the erythroblast and has a very clear distinctive blue cytoplasm in Wright's blood-stain. With the group of the clasmatocyte in the connective tissues, Maximow divided the cells into resting and active cells. With the group of endothelial derivatives in the blood, it is not wholly clear whether the larger or mononuclear forms are resting or are old forms. In the embryo, the large mononuclear forms appear less specifically differentiated. Both forms can be seen in the living chick to come from endothelium, the transitional cell being developed specifically along the line of phagocytosis and storing of particulate, solid matter and possessing a certain type of motion of the cell *in situ* and very slow locomotion like the clasmatocyte of the connective tissue.

The Granulocytes or Leucocytes

On the third day, also, granulocytes begin, represented by the cells which are analogous to the neutrophilic myelocyte of mammals. In the chick the granulocyte with fine granules is pseudo-eosiniphilic. The first sign of the beginning of the granulocytes is that a cell appears close to a vessel which cannot be distinguished from a single angioblast. I have not found in these cells any substance stainable in neutral red except the specific granulation which stains paler than the granules of the endothelium, but am not yet entirely sure that this will be a sufficient distinguishing mark between this cell and a single angioblast. When, however, such a single cell divides, there is no longer any difficulty, because two angioblasts stay together while two granuloblasts separate. This criterion is not adequate when one has only sections, but in watching the living membranes or in studying them after fixation, where every cell of an entire area can be seen in its relations to other cells, it is sufficient. Such material has obvious advantages over sections. Thus, from one cell comes a clump of four or more cells with a dense azurophilic cytoplasm, the stem cell of the monophyletic school, lying near a vessel. These cells then show the following changes. The nuclei become excentric, while the center of the cell is occupied by the centrosphere made very obvious by the development of fine granules, staining pink in neutral red, always arranged in a crescent around the centrosome. Thus, there is a nucleus on one side, a clear spot in the center of the cell and on the other side this crescent of fine granules. The granules are entirely motionless at the start, there is none of the active streaming of the granules which is always associated with amœboid movement and which must be associated with a fluid state of the cytoplasm. The cell itself, however, does move, but very slowly, directly toward the vessel. One of the cells reaches the wall, half-way between two endothelial nuclei, and then one can see the wall bend inward, until finally the cell enters the lumen. The rest of the clump line up behind the first and also pass in. Thus, these granulocytes show a specific chemotactic reaction at once. Throughout these early

stages the granules are arranged characteristically around the centrosome. Thus, the specific granulation of the red cell is arranged around the nucleus, of the granulocyte around the centrosome, while the granules of the endothelial cell are scattered throughout the cell. Even in these early stages the nuclei of many of these cells become indented, the concave side always being toward the centrosome; thus, the primitive cell may soon be regarded as a leucocyte.

In the case of the monocytes and the clasmatocytes, both of these cells can be readily found differentiating and dropping off from the endothelium, but no relation to endothelium can be made out in the case of the granulocytes. They are near vessels but never form a part of their wall. It was shown by Danchakoff[6] in 1908 that the granulocytes are extravascular in origin.

There are no eosinophilic cells on the third day. The eosinophilic granule of the chick's blood is in long rods. During the first seven days I have seen only a few in the circulation and have not found them differentiating in the area pellucida. Probably further study will bring them out, since they are known through the work of Danchakoff[5] to develop in the area opaqua of the yolk-sac. The mast cells I have not seen at all in the first seven days, and Maximow[24] found that they develop late in mammals.

From these observations one may offer the theory that the two stem cells, the angioblast with its power to give rise both to red cells and to histiocytes in the larger sense, and the granulocyte are cells whose common ancestor is a mesenchyme cell instead of a differentiated stem cell or "hæmatoblast." In other words, the cells of the blood are not so sharply marked off from the cells of the connective tissues as to have a specific, common stem cell. At least one would have to prove that the differentiated cells which normally made the syncitial masses of angioblasts could be made to develop granulocytes. The argument for the mesenchyme cell as the stem cell, for the three distinct strains of cells which contribute to the blood, is that three such groups can be isolated embryologically and that they correspond with a functional classification. At least one may say that no common differentiated stem cell has been adequately demonstrated.

Lymphocytes

In these studies of the development of blood in a living form the account of the origin of the lymphocytes is very incomplete. The lymphocytes make a group of cells ranging in the mammal from the size of a red cell up to cells twice the size. Likewise in the chick the lymphocytes are the smallest cell. When the cell first appears, all of them are of the small size. The cell has a characteristic nucleus and its cytoplasm contains a few azurophilic granules, discovered by Michaelis and Wolff.[25] The living cell has a nuclear membrane which is more distinct than in any other cell, but that this criterion is a difficult one to go by can be realized readily in connection with the fact that all nuclei become distinct as a cell dies. The cytoplasm of the lymphocytes contains but few granules and they do not stain readily in neutral red, but can be made to by increasing the amount of the dye or the time of staining. From these facts it is less readily discriminated than the other types. The reactions of lymphocytes in tissue cultures have been described by Lewis and Webster.[19] In Wright's stain, the early lymphocytes are exactly as distinctive as in adult blood. The first forms are of the small variety. I have seen a few on the fourth day, more on the fifth and sixth. In the blood smears, they occur in small clumps. The chromatin of the nuclei is very dense and has a peculiar violet reaction with azur-eosin. I have never found any indication of their differentiation in the area pellucida, thus it may be that they form only within the embryo itself rather than in the yolk-sac. However, a more extensive study of the yolk-sac may bring them out. All of the evidence from the study of this cell in the adult is that it differentiates extra-vascularly from reticulum. The only evidence, then, of significance in these studies in regard to this cell is that it occurs later than the other two groups and hence should not be regarded as a stem cell. Thus, from these studies, I would stress the use of the three names of white cells as specific for the three distinct groups, the leucocytes, the monocytes and the lymphocytes.

In these studies it is very plain that each white blood-cell, as it first appears, is differentiated. The red cells pass through a long stage of maturation. The first megaloblasts can be told as early stages of the red cells by a specific granulation, but the cell itself passes through a long series of stages, the erythroblasts, before it is the erythrocyte of the adult blood. The monocytes are a phagocytic type, like the cells of the adult, before they leave the wall of the vessel, the endothelium itself being phagocytic; the granulocytes develop their specific type of granulation early and soon begin to be leucocytes, and thirdly, the first lymphocytes are distinctive. When, however, all of these cells begin to divide, discrimination between all of the young cells is by no means easy, as the entire history of hematology attests. From this it can be seen readily that one must continue these studies of the development of blood in these living forms, watching especially the young cells just after division in all the stages of incubation, before one can adequately master all of the types of cells that are to be seen in bone marrow. In a drop of blood taken on the third day of incubation it is possible to tell all the cells apart, later it becomes most difficult. It is the study of the maturation stages of each group of cells by means of the eosin-azur technique that has been the great contribution of the monophyletic school. To this study must now be added certain specific criteria that come out through the method of applying dyes to living cells and we must now follow the stages of the cells with these vital dyes through the different embryonic periods.

The postulation of three strains of blood-cells on the basis of embryology fits in with the functional groups as we now know them. The endothelial or angioblastic group represents first the hemoglobin-bearing cells, and secondly, that group of the blood-cells which exhibit a special property of endothelium, namely, phagocytosis. The monocytes have this power of phagocytosis, they possess a peculiar type of motion *in situ* with very slow locomotion. The granulocytes possess a high

degree of amœboid motion, with speed and a flowing of the granules. They respond to chemotactic influences, are also phagocytic and have functions probably related to their specific granulations. The lymphocyte strain, as Murphy[26-29] has shown, are separated off physiologically by their being more sensitive to X-rays and to the emanation of radium than other normal cells. Moreover, he has shown that they are related to immunity toward certain forms of tumors as well as to certain types of infection.

The study of the blood-cells is a part of the study of the cells of the connective tissues. The erythrocytes are the only type that function only within the vessels. Of the other group from endothelium, the histiocyte in the larger sense, the vast majority make the clasmatocytes of the connective tissues, which are the mononuclear forms and the actively phagocytic forms of sub-acute infection, the resting and active wandering cells of Maximow. A few of this group make the monocytes, that is, the large mononuclear and transitional forms of the blood.

Of the granulocytes, which all differentiate extra-vascularly, the neutrophilic leucocytes pass into the vessels in largest numbers. Of the eosinophiles very many remain in the tissues while the mast cells never enter the vessels in most animal forms. By mast cell is meant a cell of the connective tissues occurring along vessels, along nerves and between muscle fibers, having a special metachromatic, basophilic granule. The so-called mast cell of human blood has been shown by Weidenreich,[39-41] to be a degenerating cell without any centrosome. The lymphocytes are for the most part extra-vascular, arising in the lymph glands and in the follicles of the spleen and in very numerous follicles in the various organs either associated with lymphatic capillaries or not. Thus, the differentiation of three strains of blood-cells, the endothelial strain, the granulocyte strain and the lymphocyte strain, that can be made out in the early stages of the chick embryo, can be shown to correspond with a functional grouping as far as we yet know the functions of the types of blood-cells. Moreover, the origin of the cells of the blood can be shown to be but a part of the study of the great groups of wandering cells of the connective tissues, the one type which functions only intra-vascularly being the erythrocyte.

The method of studying blood with vital dyes, beginning with the stages of the embryo when the cells first appear gives a very great advantage in following the maturation of specific cells and gives a chance of analyzing the complicated young forms which it is necessary to recognize in order to understand bone marrow.

The group of the red cells is characterized by a specific granulation stainable in certain vital dyes, possibly one should say precipitated by these dyes. This substance is at first throughout the cytoplasm, then in a wreath around the nucleus, then a reticular form and finally in scattered granules or droplets. The arrangement of this granulation around the nucleus should be stressed, although the substance is of cytoplasmic not of nuclear origin. Red cells with nuclear fragments, Howell-Jolly bodies, can be shown to be dying cells. The strains of white cells, clasmatocytes and monocytes, that come from endothelium, are characterized by certain granules and vacuoles stainable in very dilute neutral red. They are scattered diffusely throughout the cells. The granulocytes are characterized by the arrangement of their specific granulation with reference to the centrosome. The lymphocytes are less sharply characterized morphologically, but have somewhat distinctive nuclei and granules stainable in azur.

This work is a part of the new subject of experimental cytology which seeks to analyze cells by means of specific criteria and to use these criteria to study the reactions of cells to normal and abnormal conditions.

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SOME OXIDATION MECHANISMS OF THE CELL[1]

By F. Gowland Hopkins

Oxidation in the living tissues offers to biochemistry what is perhaps its chief problem. The living cell receives a supply of molecular oxygen; it also receives materials which it ultimately oxidises. Yet towards these materials molecular oxygen is indifferent. These facts define the problem. The biochemist cannot avoid the task of trying to solve it.

I propose to devote two lectures to a consideration of certain limited aspects of the subject, feeling sure that you will understand the necessity for limitation. On the present occasion I shall deal with a special type of chemical mechanism, concerned directly or indirectly with oxidations in the cell, which has been the subject of recent investigations. The mechanism in question is found in nearly all animal tissues, and is active in certain tissues of the plant.

The second lecture will deal with a highly specialised tissue—striated muscle. It will be devoted to a discussion of the incidence of oxidations; to their position, that is, in a chemical sequence. Incidentally it will deal with the influence of oxygen upon equilibrium in the tissue. A prominent point in all that I shall have to say is the importance of the events which precede actual oxidations.

It is always desirable, before dealing from any standpoint with the functions of oxygen in living processes, to have in mind the fact that life in certain associations can dispense with it altogether. We know that certain living organisms, usually of a kind adjusted to the conditions of parasitism, are maintained without oxygen, and among them are not micro-organisms alone but highly differentiated animals, such as intestinal worms. The energy requirements of such animals are met by the occurrence of exothermic chemical processes which involve the employment of free molecular oxygen. Though it is quite possible that life began in the sea at a time when the available oxygen in the atmosphere was much less than at present, we are perhaps not justified in assuming that these living processes without oxygen are more primitive than those in which it is employed. Nevertheless, the functions of oxygen in life do represent something superadded to such phenomena as are entirely essential. It is important to the line of thought which you will be asked to follow that two aspects of this relation should be emphasized. The first is that living tissues, even those of the higher animals which are fully adjusted to employ oxidations to advantage, do not necessarily lack the power of extracting energy from materials in the absence of oxygen. Such methods of obtaining energy—this being the second point for emphasis—are, however, relatively, extravagant. Perhaps the most important function of oxygen in living processes is to establish economy of material.

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Much intellectual ingenuity has been expended in the endeavor to throw light on the oxidative mechanisms of the cell and not a few theories have been propounded. Those which, from one standpoint or another, have visualised the activation of molecular oxygen or the influence of special catalysts within the cell have stimulated experiment and assisted progress, even when they have, as theories, failed to survive.

Certain views, however, which were developed during the last third of the nineteenth century have, in my opinion, inhibited productive thought and discouraged experiment. I allude to those views which picture the dynamic events associated with life, including of course the essential oxidation, as occurring in a particular kind of large chemical molecule within the cell. During phases of construction or reconstruction, both the oxygen and the materials to be oxidised are supposed to be built up into the structure of this living molecule. Alternating with such phases, and particularly encouraged by stimulation, are phases of breakdown during which the products of oxidation emerge from the molecule, accompanied by liberated energy. Such views were expressed vaguely in the *inogen hypothesis* of Hermann and more objectively in Ehrlich's *side chain theory* and Verworn's *biogen hypothesis.* All these imply that those parts of a living cell which manifest the essential attributes of life are made up of a number of like molecules, each of which is in some sort a unit of living matter. When they remain quite vague in detail, such conceptions offer no aid to thought; when details are supplied, they attribute qualities to the living unit which can hardly belong to a single molecule, if we use the word in any ordinary sense as denoting a system in which atoms or atomic groups are associated by valency.

The alternative view is that the living cell (or an equivalent structure) is itself the unit. The manifestations of life viewed from this standpoint depend upon changes undergone by diverse molecules of a kind which need not elude ordinary chemical studies. Such molecules, of an order common to other non-biological chemical events, take part in diverse chemical reactions which, though they occur in a colloidal milieu, are themselves ordinary in the sense that they can—individually at any rate—be studied by the recognized methods of chemistry. On this view, the essence of what is peculiar to the cell as a chemical system lies not in the nature but in the organization of its processes.

To explain the remarkable organization called for, is, and doubtless will long remain, a very hard task. Nevertheless, the view just mentioned is the one which is supported by all the experimental facts that we possess, and is one which will most surely stimulate further experimentation. It is to be observed of all theories based upon the conception of a living molecule or biogen that they make no real attempt to explain the unknown in terms of the known. They escape from the hard task of analysis by falling back upon the properties of an entity to which, since it is wholly imagined, any attributes may be ascribed. They form, I think, little more than comfortable couches whereon the mind may rest and forget the task which after all remains ahead.

It seems to me that the two opposed views that are before us, the one conceiving that all the chemical events in the cell occur within the structure of a particular and highly exceptional kind of molecule, the other looking to the progress of ordinary chemical reactions, with associated changes in the physical equilibrium of colloid systems, as being the essential basis of events, may be tested by the application of one particular criterion. The former view, if it is to help thought at all, involves inevitably a belief in intramolecular oxygen; in the building up of oxygen into the living complex upon which it confers instability. If oxygen be not "assimilated" before it functions; if, that is to say, the oxidations in living tissues proceed under the influence of an immediate and contemporary oxygen supply, then it is sure the biogen hypothesis loses almost the whole of any value it might otherwise possess. I shall deal later—more particularly in my second lecture—with evidence which provides this criterion. It leaves no doubt in my opinion that the conception of intramolecular, assimilated, oxygen is without basis in biology.

I will ask you to believe that this preliminary reference to considerations which are vague is not without point. Had we to admit that conceptions such as that of the living biogen are in any sense necessary to biological thought, I personally feel that the experimental findings which are to form the main theme of this lecture, and all others of a similar order, would lose most of their significance. They would refer only to events in the cell which are purely superficial. The really significant events occurring in an unapproachable molecule would evade such experimental attacks altogether. I am to deal with certain special facts alone, but in order that these may fit into their proper place it is necessary for me first to go over certain more familiar ground.

It is right to speak of Schönbein as the real pioneer in experimental attacks upon our problem. This distinguished observer (I am quoting the late J. H. Kastle) seems to have been the first to recognize clearly that in the final products of chemical combination, particularly those in which oxygen is concerned, we see, as he puts it, only the closing scene of a chemical drama which is in reality composed of several intermediate acts, and that for a correct understanding of such processes it is quite as essential to know these intermediate acts as it is to know the beginning and the end. All illuminating studies of metabolism have been inspired by this belief.

The study of tissue oxidations thus received its impetus under the influence of Schönbein in the middle of the last century and, since he wrote, it has on one line or another continued. But really significant data leading to modern views may be said to have first arrived with studies of autoxidation as made by Engler and Bach and extended into biological problems by Bach and Chodat. The accepted views based upon the work of these authors were foreshadowed by Traube, though upon small experimental basis.

Certain substances—though neither the foodstuffs nor their immediate derivatives—are capable of spontaneous oxidation when in contact with molecular oxygen, and this at ordinary temperatures. When such autoxidation occurs it can be

usually shown that the oxygen is taken up, not atom by atom, but as a molecule still partly intact, only one of the two bonds of the original molecule being opened up. Thus if R represent the autoxidisable substance there is first formed an association of the peroxide type, $R\!<\!\begin{smallmatrix}O\\|\\O\end{smallmatrix}$. Ostwald and Bach have pointed out that, as a matter of fact, such a compound must, under the circumstances of autoxidation, be in all cases first formed, even if it have but an evanescent existence. By Ostwald's Law of Successive Reactions the less stable state represented by RO_2+R must precede the more stable state $RO+RO$.

If autoxidation is thus always associated with peroxide formation, its occurrence in the cell even on a very small scale must be a significant thing, because once peroxide oxygen has appeared it may be transferred to substances not themselves autoxidisable. Autoxidisable substances can act catalytically or as parts of a catalytic system. If such are present in the cell, however low their concentration, we have at least the beginnings of a mechanism for tissue oxidations. I need hardly remind you that an amount, probably minute, of material capable of autoxidation is known to exist in living tissues, or at least in some of them. Endowed with the power of combining with atmospheric oxygen to form peroxides, such substances constitute the so-called oxygenase factor in oxidase systems. A second factor in these systems—the peroxidase—in some way accelerates the transference of the peroxide oxygen to substances by themselves incapable of taking up the original atmospheric oxygen. The nature of peroxidases and how they act need not trouble us here. We may rest content with the close analogy between their influence on the activity of organic peroxides (oxygenases) and that of iron on oxidations by hydrogen peroxide.

These oxidase systems are doubtless familiar to you, and I need discuss their nature no further, though I would like to remind you of an interesting fact which we owe to my colleague at Cambridge, Mrs. Onslow [2] (formerly Miss Muriel Wheldale). She has shown that the autoxidisable substance which functions as the oxygenase in many plant tissues is catechol, or substances containing the catechol grouping; the ortho relation of two hydroxy-groups in these compounds makes them prone, apparently, to peroxide formation.

The oxidations which have been observed to occur under the influence of these oxidase systems are not such as involve deep-seated change or massive energy discharges in the cell. They have been thought of as being mainly protective, rather than metabolic, in function. It would be wrong, however, to forget that their activity as observed in the test-tube may be more limited in its effects than when it is displayed as part of an organised sequence of events within the cell. It is sure that cell oxidations involve the organization of various agencies. The recognition that one of these agencies secures the availability of peroxide oxygen undoubtedly constitutes a step towards the solution of the problem with which we are dealing.

But I am more concerned to remind you of other agencies. Oxidations may occur without the aid of atmospheric oxygen.

In a sense it is true to say that every oxidation is associated with reduction. Any agent which oxidises is itself reduced. Sometimes the reduction process seems to be the essence of the phenomenon, as when hydrogen is transferred from one substance to another; the latter being thus reduced. By long established custom, however, and with full justification we speak of the substance which loses hydrogen as being itself oxidised. I would not refer to so elementary a point were it not so important in all that follows.

The simultaneity of oxidation and reduction may present a prominent aspect of the phenomena when the molecule of water is concerned in chemical changes. It is of much biological importance to realize that oxidations and reductions in the living cell may follow upon a simultaneous transference of the ionic components of the water molecule. Water after all is by far the most abundant reagent present in the cell. In following up this aspect of events we shall be considering tissue oxidations which proceed, up to a certain point at least, without the intervention of atmospheric oxygen.

It will be convenient here to note a point in nomenclature. Engler gave the name of *oxygen acceptor* to any substance which may take up the oxygen of peroxides formed in autoxidation. When reduction and oxidation occur together, as in the case we are about to consider, we speak on the one hand of the *hydrogen acceptor* and on the other of the *oxygen acceptor*. The terms are convenient.

Bach [3] has very fully studied reactions which he calls *hydrolytic oxidation-reduction reactions*. In these under the influence of such catalysts as metals of the platinum group the reduction of one substance by the hydrogen ions of water is associated with the simultaneous oxidation of another substance by the hydroxyl ion. Such a case, with an organic catalyst taking the place of the metal, is the well-known Schardinger reaction induced by an enzyme present in fresh milk. Milk neither reduces methylene blue nor oxidises an aldehyde, but if both are present, the former substance acts as a hydrogen acceptor and the latter as an oxygen acceptor, and simultaneous reduction and oxidation occur. Bach has shown that in the tissues there is an enzyme identical with, or similar to, the Schardinger ferment of milk. It is important to remember that in reactions catalysed by enzymes of this class—at any rate if Bach's view concerning their action be correct—a hydrogen acceptor and an oxygen acceptor must both be present. It should be noted that Schardinger's ferment plus an oxygen acceptor would constitute a system with the properties of a *reductase* or *reducase*. On the other hand, combined with a hydrogen acceptor the ferment would show itself as a catalyser of anaërobic oxidations.

[2] Muriel Wheldale Onslow: Bioch. Jour., 1919, XIII, 1, *Ib.*, 1920, XIV, 535 and 541.

[3] Bach: Berichte, 1909, XLII, 4463; Oppenheimer's Handbuch, Ergänzungsband, 1913.

Having just now referred to the use of methylene blue as a hydrogen acceptor I should like to add just a word concerning it.

In classical experiments, Ehrlich, as you are well aware, injected into the living animal dyes of this class, which became colorless on reduction, to test the oxygen affinity of the tissues. The method as used by him has suffered much criticism of late, and the results it yields are doubtless open to misinterpretation. But, like other technical methods liable to error in uncontrolled experiments, it may yield valuable evidence when a proper control is provided. Such an informal use of methylene blue injections was made by the late Dr. Christian Herter[4] when he showed that depression in the temperature of the body diminishes the intensity of oxidations in the nerve cells of the brain, while fever increases it. It is a source of satisfaction to know that the facts I am to put before you are very closely related to matters which awakened interest in Dr. Herter's mind during the later years of his scientific life.

I shall have to refer a good deal to the use of methylene blue; but only when employed in the test-tube as an indicator of the fact that labile hydrogen is present in a particular chemical system. When the dye is decolorized we know that hydrogen has been transferred to its molecule. We assume, generally with full justification, as we register the progress of decolorization, that the same reduction will happen in the cell when a suitable physiological acceptor of hydrogen is present. The dye is merely a representative of such.

Returning to the tissues I must next point out that Wieland[5] claims for them the possession of a catalytic mechanism of which the influence may be general enough to include the supposed work of those already mentioned.

This author sees the essential cause of certain typical biological oxidations in the activation, not of oxygen, but of hydrogen. A proof that this activation occurs would render unnecessary, he thinks, any attempt to give a general application to the Engler-Bach peroxide theory, while it should modify somewhat the angle from which the hydrolytic oxidation-reduction processes are viewed.

Starting with the conception of a catalyst which acts as a "dehydrase" and confers lability on hydrogen atoms, the assumption that oxidases, reductases and "mutases" separately exist becomes from Wieland's standpoint superfluous.

His conceptions are especially supported by a careful quantitative study of the action of Schardinger's ferment upon salicylaldehyde.

The aldehyde is assumed to act in the form of its hydrate. The enzyme activates two of its hydrogen atoms which are then made available for an acceptor. This acceptor may be (1) molecular oxygen, (2) such reducible substances as are represented by methylene blue, or, in a particular case the acceptor may be (3) a molecule of the aldehyde itself in its unhydrated form. As an end result we have, in case (1), a frank oxidation of the aldehyde; in case (2), an observed reduction which, however, is associated with indirect oxidation of the aldehyde; in case (3), on the other hand, the Cannizzaro reaction—the so-called "mutase" effect.

The following scheme illustrates these relations:

$$R-CH\begin{matrix} \diagup OH \\ \\ \diagdown OH \end{matrix} \quad \left.\begin{array}{lll} \xrightarrow{1} & H_2 & O=H_2O \quad + \\ \xrightarrow{2} & H_2 & A=AH_2 \quad + \\ \xrightarrow{3} & H_2 & R.COH=RCH_2OH + \end{array}\right\} R.COOH$$

Whether any of these end results is attained, or a mixture of all three, depends—as Wieland shows—upon the concentration, and speed of reaction of the various acceptors. The most important hydrogen acceptor for energy production in the cell is, clearly, oxygen; but the reduction of other acceptors may be equally important for other aspects of cell activity.

I do not myself think that the existence of a catalyst capable of securing the shifting of hydrogen on these lines should be thought to lessen the importance of those oxidase systems which we earlier considered. After all there is direct evidence for the formation of peroxides in the tissues, and in the organization of events it is likely that more than one mechanism is used, even to attain what may seem, superficially, to be similar results. Wieland, as we have seen, assumes in the particular case which serves as an examplar of a "dehydrase" reaction, that the aldehyde reacts as a hydrate. The necessity for this assumption shows after all that the special action of the enzyme as conceived need not differ in essentials from the hydrolytic oxidation-reduction of Bach. Wieland's experiments are very instructive, however, and I have found his views to be very helpful in dealing with facts which I shall later put before you.

Thunberg[6] has accepted Wieland's conceptions and in a paper recently published he has used the methylene blue technique in striking experiments which supply evidence—as he believes—bearing on the probable nature of intermediary metabolites. He suspended muscular tissue, deprived of its power of reducing methylene blue by thorough washing with water, but still containing the catalyst, in a weak solution of the dye. Small quantities of the substance under investigation were then added. The methylene blue acted, of course, once more as the hydrogen acceptor. If the second substance were capable under the influence of the enzyme of yielding hydrogen, acting, that is, in Thunberg's terminology as a hydrogen "donator," then, the system being completed, the dye was decolorized. Thunberg speaks of the ferment as a hydrogen "transportase." The substances which proved themselves to be donators were certain organic acids like succinic, malic, lactic, oxybutyric, and others; also amino acids like alanine and glutamic acids. They formed only a limited proportion of the large number of substances tested, and the fact that their structure evidently fitted into the system established by the tissue ferment and a hydrogen acceptor yields, in Thunberg's view, presumptive evidence that they represent actual tissue metabolites. The technique of these experiments was a little less simple than my description would imply. There was evidence of the existence of specific catalysts, each

[4] Herter: Amer. J. Physiol., XII, 128.

[5] Wieland: Berichte, 1914, XLVII, 2085.

[6] Thunberg: Skand. Arch. Physiol., 1920.

related to one substance rather than to others and, in a given case, the tissue might have to undergo preliminary treatment in order to isolate more or less the activity of the particular enzyme concerned.

I have dealt rather fully with this recent work which seems to establish the importance of the conception of acceptors, and especially of hydrogen acceptors when they co-exist with substances tô be oxidized. I have done so because the facts, if not the views, involved prepare the way, I think, for the presentation of some experimental results obtained by myself which I wish to put before you. You must not judge of the significance of the doctrine of the activation of hydrogen without remembering that it supposes the ultimate fate of the hydrogen, labilized and transported, is to be itself oxidized. It ultimately, if not immediately, appears in such form or relations that it becomes amenable to oxidation by molecular oxygen. It is interesting to note that these recent views involve a certain divagation in the paths, and a difference in the mechanisms whereby carbon and hydrogen are respectively brought to oxidation. If, to take a single merely illustrative instance, lactic acid be supposed to act first as a hydrogen donator, and Thunberg's observations seem to show that it can so act, the loss of its labile hydrogen would convert it into pyruvic acid.

$$CH_3CHOH.\ COOH \longrightarrow CH_3.\ CO.\ COOH + H_2.$$

The hydrogen if not oxidized straightway will be transferred to some acceptor on its way to oxidation, whereas the first evolution of CO_2 from the original lactic acid molecule must involve a quite different type of change. It may, for instance, be evolved from pyruvic acid under the influence of a carboxylase—such catalysts being known to exist in animal tissues. The removal of hydrogen from saturated molecules may, in the cell, be a necessary preliminary to such a process as this, as well as making the carbon chain more open to the attack of molecular oxygen.

I now come to the main purpose of this lecture which is to describe an actual tissue constituent susceptible of autoxidation and able to play a part either as an acceptor or donator of hydrogen.

In 1888 de Rey-Pailhade[7] showed that yeast cells and aqueous extracts of yeast have the property of reducing sulphur to hydrogen-sulphide. Later he showed that many animal tissues possess the same property. Throughout a long series of communications upon the subject he has courageously maintained the view that the labile hydrogen thus shown to exist in living cells has important respiratory functions. His views as to the probable nature of the hypothetical substance which he supposed to carry this labile hydrogen and to which he gave the name *Philothion,* have been modified from time to time. In his latest writings he speaks of it as the hydride of a protein ("Hydrure d'albumine"). After the publications of Heffter, to which reference will immediately be made, he accepted the view that the labile hydrogen exists in sulphydryl groups, HS—.

[7] Comptes rendus, 1889.

In 1901 Mörner employed the delicate nitro-prusside reaction for the identification of cysteïn, and a little later Gola found by its use that a substance which presumably contains the HS— group, is characteristically present in proliferating plant tissues. Buffa then showed that the same color reaction is given by certain animal tissues.

In 1908 Heffter[8] applied the nitro-prusside test to a great number of tissues and tissue extracts and obtained positive results in many cases. Later, it would seem independently, V. Arnold[9] showed that the reaction under proper conditions is displayed by practically all organized animal tissues. He at first described it as a protein reaction, though recognizing that the plasma proteins gave a negative result.

A little later Arnold found that a strong nitro-prusside reaction may be given by protein-free extracts of tissues, and came to the conclusion that free cysteïn was the responsible substance. Although he did not isolate cysteïn, he considered that the evidence proved it to be a primary cell constituent in Kossel's sense.

Heffter, however, first gave definite form to the view that the presence of the HS— group, whatever its associations, may, owing to its labile hydrogen, be, in part at least, responsible for the reducing properties of protoplasm, and also, perhaps, indirectly for oxidations in the cell. Basing it upon known analogies he made the suggestion that during the autoxidation of the sulphydryl group pure oxide of hydrogen may be formed, and the peroxide oxygen then transferred, with or without the influence of a peroxidase, to other substances in the cell. If, further, HS— groups can be supposed to act continuously as promoters of cell oxidations, their own spontaneous oxidation to —S—S— groups must be reversible. In this connection Heffter called attention to the fact that cystine can be reduced to cysteïn by sodium sulphite, and suggested that in the cell some substance might, like the sulphite, act as an "acceptor" for the oxygen of the water molecule, leaving the hydrogen of this to reduce the di-sulphide group once more to sulphydryl groups. Heffter's views have become generally familiar owing to the publication of an admirable discussion by Thunberg,[10] in 1911, of the possibilities which underlie them.

Though very sugestive, these views have remained wholly theoretical. No experimental proof has been given of the existence of free cysteïn in the cell in the cells pictured by Arnold, nor has an HS— group been located in any other substance. Not without an isolation of the cell constituent actually responsible for the nitro-prusside reaction, and a study of its properties, could Heffter's theoretical views receive experimental support.

Such a substance I have recently succeeded in isolating and I propose to give you some account of it. To judge from color reactions it is present, I think, in all active animal tissues and probably in such vegetable cells as are engaged in the processes of growth and subdivision. It has so far been

[8] Heffter: Medicinisch-naturwissensch. Arch., 1908, I, 81.

[9] Arnold: Zeitsch. f. physiol. Chem., 1911, LXX, 300 and 314.

[10] Thunberg: Ergebnisse d. Physiol., 1911, XI, 328.

actually isolated from yeast, from striated muscle, and from mammalian liver alone. In them it is certainly responsible for the nitro-prusside reaction of the fresh tissue. The three cases are so diverse that it becomes highly probable that the color reaction as given by other tissues is due to the same substance.

It is present in very low concentration; perhaps to the extent of not more than 0.02 per cent of the fresh tissue. The significance of a tissue constituent is not, however, to be measured by the concentration in which it occurs. This is a point on which it is well to insist in connection with chemical studies of living tissues. A substance found in very small amounts may have a metabolic importance as great as that of another substance found in relatively large amounts. Many chemical reactions in the body form part of an ordered series. They are successive reactions. Now if one substance be changing into another through a series of intermediate products, then as soon as dynamic equilibrium has been established in the series—and to such equilibrium tissue processes always tend—the rate of production of any one intermediate product must be equal to the rate at which it changes into the next, and so throughout the series. But the rate of chemical change is measured by the product of the efficient concentration of the substance undergoing change and the velocity-constant of the change in question.

If K_1, K_2, K_3, K_4 are the respective velocity-constants of successive reactions in equilibrium, and [A], [B], [C], [D] are the corresponding concentrations of the substance we must find:

$$K_1[A] = K_2[B] = K_3[C] = K_4[D] = \text{etc.}$$

It follows that if K_1 is small and K_2 large, [A] must be large and [B] small and so on; clearly the significance of B is from any point of view no less than that of A. In the case, moreover, of the substance having catalytic functions, general experience leads to the expectation that it may be efficient as an agent though present in very low concentration. In certain measure such functions are attached to the substance to be now described.

You will not wish to be troubled with the technical details involved in the process of its isolation. It has shown itself to be a dipeptide containing cysteïn and glutamic acid. How the two amino-acids are linked in the molecule has not yet been determined with certainty but preliminary studies suggest that the dipeptide is cysteïnyl-glutamic acid:

$$HS\cdot CH_2\cdot CH\cdot NH_2\cdot CO-NH\cdot \underset{\displaystyle COOH}{\underset{|}{CH}}\cdot CH_2\cdot CH_2\cdot COOH.$$

I hope to convince you that the HS— group of the cysteïn component has dynamic functions within the cell. The reason that this group with its functions is found in a dipeptide of cysteïn rather than in free cysteïn itself is probably associated with the fact that for some reason the former is more resistant to the destructive changes of metabolism. The dipeptide is autoxidisable. When in neutral or slightly alkaline solution, it spontaneously oxidizes in the air at ordinary temperatures to form a compound with a disulphide linking on lines which are common to substances containing a sulphydryl group.

$$2(R-SH)+O=R-S-S-R+H_2O.$$

By various means it is possible to reduce the oxidized compound back to its original form. The HS— group of the dipeptide acts, one may say, as a hydrogen donator while the —S—S— group of its oxidized form acts as a hydrogen acceptor. The full significance of these properties only comes to light, however, when it is shown that the oxidation and reduction (or the removal and addition of hydrogen) are brought about in a reversible manner by factors present in the living cell itself. That fresh tissues possess a reduction potential such as to induce rapid reduction of the substance can be at once shown by the simplest technique. In one test-tube are placed a few cubic centimeters of distilled water and in another an equal amount of a weak solution of the dipeptide in its oxidized form, which gives of course no nitro-prusside reaction. A small weighed piece of fresh tissue (liver, kidney, muscle etc.) is then dropped into each. If the tubes be then placed in a bath at 35° C., it will be found that the fluid of that containing the dipeptide will, after an hour or two, give the nitro-prusside reaction with an intensity out of all proportion to the fluid of the control tube. The latter may give a slight reaction due to diffusion from the tissue of preformed reduced dipeptide. Under anaërobic conditions the change goes faster. Antiseptics may be used. The reduction may be followed in its stages by placing crystals of ammonium sulphate mixed with nitro-prusside on a tile. A couple of drops of the fluid followed by one of ammonia will show the increasing intensity of the colour reaction. It is difficult to obtain the reducing system in solution; but the dry powder, obtained by grinding a tissue with sand under alcohol, washing the residue with fresh alcohol on a filter, and allowing it to dry in the air, reduces efficiently. Such a powdered preparation in the case of liver, deprived as far as possible of connective tissue, has served well for quantitative observations.

It is next easy to show that conditions exist, on the other hand, in which factors present in tissues can promote oxidation of the reduced dipeptide. It is a circumstance familiar to all who have studied the reducing power of excised tissues, by the use, for instance, of methylene blue as an indicator, that the reduction potential rapidly falls off as survival processes progress. It must of course be remembered that conditions for reduction and oxidation vary with the substance concerned. In the case of the dipeptide survival changes involve a complete reversal of the existing relations. A tissue which has stood under aseptic conditions for a sufficient time fails to exhibit the nitro-prusside reaction, and it can be shown without difficulty that conditions then develop under which the reduced dipeptide is oxidized. As this oxidation goes on anaërobically some other substance must now act as a hydrogen acceptor. Especially upon actual autolysis does this change in the equilibrium of reducing and oxidizing agencies occur. If disintegrated tissues be suspended in four to five times their weight of chloroform water and allowed to auto-

lyse in corked flasks at 37°, the fluid during the first few hours will show a strong nitro-prusside reaction. This gradually fails in the fluid, and, ultimately, after a period varying with the particular tissue, also in the incompletely autolysed tissue fragments. The end point occurs soonest with kidney; more slowly with liver, because of its original high content of the reduced dipeptide; and still more slowly in the case of muscle. If when the color reaction has ceased a further quantity of reduced dipeptide be introduced so as to restore a strong reaction to the fluid, this will be found also to disappear on standing. The process goes on with undiminished velocity, and indeed, seemingly faster, under anaërobic conditions. That oxidation has occurred is shown by the fact that the process is easily reversible by reduction. If some of the fluid which has ceased to give the color reaction, and especially if its content of dipeptide had been increased on the lines mentioned, be boiled, filtered, and placed when cool over a piece of perfectly fresh tissue, an intense nitro-prusside reaction once more develops. The significance of this change of oxidation potential during survival and post-mortem changes need not be here discussed. For the moment I am concerned only to show that these exist in tissue mechanisms for both reduction and oxidation of the dipeptide. If, *in vitro*, the one has to disappear before the other can be displayed, it is yet perfectly probable that in the geographical organization of the cell both mechanisms are employed.

Considerations may now be put forward which indicate that the dipeptide does as a matter of fact play a real part in cell dynamics. Fresh tissues, of course, reduce methylene blue and so when itself in the reduced condition does the dipeptide. But as has just been shown, the former also reduces the latter. It would seem then that a mechanism in the tissues has a greater reduction (or lower oxidation) potential than the HS– group of the dipeptides. As a matter of fact, however, the relations depend upon the hydrogen-ion concentration of the medium. It should be remarked that the reduced condition of the dipeptide is more stable on the acid side of neutrality. The following facts are of interest.

If the substance in the oxidized (disulphide) form be added to a solution of methylene blue in contact with fresh tissue, then if the reaction of the system is even slightly on the acid side of neutrality, say at pH = 6.8, the reduction of the dye is greatly slowed, as the control preparation will at once show. The dipeptide acts here simply as a hydrogen acceptor and competes with the methylene blue in this respect, delaying or (according to its concentration) even preventing the reduction of the latter. If, however, the reaction of the medium be adjusted to pH 7.4, or made very slightly more alkaline than this, the relations change. The normal rate of reduction of methylene blue by the tissues used is then markedly *accelerated* by the addition of the *oxidized* dipeptide. The phenomena are best observed under anaërobic conditions and the observations should be made in test-tubes which can be evacuated or which are corked and so fitted with glass tubing that the air can be replaced by pure nitrogen. As the tissues contain their own supply of the substance the contrast between the behavior of a preparation to which dipeptide has been added and a control preparation is greater if the tissue used is first washed several times with sterilized distilled water. This removes a considerable part of the pre-existing dipeptide especially from the surface of the tissue. The tissue powder from alcohol, as described above, acts well and may also be washed before use.[11] Although the fact is not commented upon by authors who have used washed tissues in connection with methylene blue reduction, the concentration of the enzyme is reduced by extraction with water. In the experiments under discussion washing increases the contrast, but may increase the actual time required for reduction.

The following data will illustrate what has been said. In the experiments given the tissue preparation was in each case the liver of the rat, ground under alcohol, air-dried, and afterwards washed with distilled water. Similar results have been obtained, however, with other tissues washed and unwashed. The first comparison shows the reversal in the effect of added dipeptide when the reaction of the system passes from acid to neutral or slightly alkaline. The tubes were filled with nitrogen:

Tissue preparation	Oxidised dipeptide added	Me. blue 1:5000	Water	pH at beginning	Reduction time
0.5 gms.	0	0.5 c.c.	5 c.c.	7.5	3 hrs. 35 mins.
0.5 gms.	4 mgms.	0.5 c.c.	5 c.c.	7.5	1 hr. 30 mins.
0.5 gms.	4 mgms.	0.5 c.c.	5 c.c.	6.8	5 hrs. 20 mins.
0.5 gms.	8 mgms.	0.5 c.c.	5 c.c.	6.8	15 hrs. +

The following shows the acceleration of reduction due to the addition of oxidized dipeptide to the system in neutral or slightly alkaline conditions. The solution in each case was a phosphate buffer solution, the pH being adjusted after the addition of the dipeptide. Chloroform was added to all the tubes and there was a layer of toluol on the surface. The tubes were filled with nitrogen.

Tissue preparation	Buffer solution	Me. blue	Oxidised dipeptide added	pH	Reduction time
0.2 gms.	5 c.c.	0.5 c.c.	0	7.4	5 hrs. 45 mins.
0.2 gms.	5 c.c.	0.5 c.c.	4 mgms.	7.4	2 hrs. 40 mins.
0.2 gms.	5 c.c.	0.5 c.c.	0	7.8	3 hrs. 45 mins.
0.2 gms.	5 c.c.	0.5 c.c.	4 mgms.	7.8	1 hr. 40 mins.
0.5 gms.	5 c.c.	0.5 c.c.	0	7.8	2 hrs. 30 mins.
0.5 gms.	5 c.c.	0.5 c.c.	4 mgms.	7.8	0 hrs. 55 mins.
0.5 gms.	5 c.c.	0.5 c.c.	8 mgms.	7.8	0 hrs. 22 mins.

It seems clear that in the reactions described the –S–S– group of the dipeptides acts first of all as a hydrogen acceptor

[11] It is noteworthy that the tissues of small animals such as the rat show under all circumstances a greater reducing power than those of larger animals.

and under conditions of even slight acidity the resulting HS— groups are too stable to transfer the hydrogen to another acceptor. In neutral or slightly alkaline solutions, on the other hand, the hydrogen is transferred to the methylene blue. If this view be the right one, we have to recognize the important fact that the two reactions involved in the transference of hydrogen to the disulphide group under the influence of a tissue enzyme and its subsequent transference from sulphydryl groups to the methylene-blue acceptor, together run faster than the single reaction in which the dye is directly reduced by the tissue enzyme. The dipeptide then possesses what are essentially catalytic properties and could be fairly spoken of as a co-enzyme. Indeed, if tissues are very thoroughly washed, as in the observations of Thunberg referred to earlier, so that their power to reduce methylene blue is practically removed, the restoration of this power when the *oxidized* dipeptide is added at once gives the impression that it has the function of a co-ferment exerted on the lines suggested. But Thunberg's work suggests, of course, another possibility. He found, as already stated, that a number of substances such as succinic acid, malic acid and the like, including, as is noteworthy, glutamic acid, can under defined conditions restore reducing power to tissues deprived of it by washing. It is possible, therefore, that the dipeptide in the experiments just described was, when in neutral or alkaline solution, merely acting as a substance of this class. I am convinced, however, that this is not the case. The compounds which acted as hydrogen "donators" in Thunberg's experiments were undergoing an irreversible oxidation due to the removal of hydrogen atoms attached to carbon. I have evidence to show that this does not occur in the case of the dipeptide. Oxidation and reduction of this substance by tissue systems involve the thio group alone and the strictly reversible change:

$$-SH \quad HS- \rightleftarrows -S-S- + H_2.$$

The fact that the living cell actually contains a substance with the properties described gives strong support to the belief that the activation and transport of hydrogen represents one aspect of the chemical dyamics of tissues. It is probably an important aspect, though only a part of the manifold phenomena of biological oxidations. It would certainly be wrong to look upon it as more than partial. It is the duty of the bio-chemist to study as fully as possible every special line of chemical change within the living cell that he is fortunate enough to recognize and define. But he must remember that to relate each such line of change with the many others which accompany it—to study, that is, the organization of reactions in the cell—is a task which lies ahead.

STUDIES ON SOME OF THE NON-LIPOID COMPONENTS OF BLOOD SERUM IN RELATION TO ITS ANTIHEMOLYTIC PROPERTY

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In an earlier paper [1] it was reported that the protective power against hemolysis of guinea-pig corpuscles by sodium oleate and saponin is diminished in the serum from cases of pernicious anæmia and those in which the liver and spleen are implicated. Because of this finding it seemed worth while to study the substance or substances in the serum upon which the protective power against hemolysis depends. Direct experiments with lecithin and cholesterin, alone and in combination, did not yield evidence that either one is the sole substance in question.[2] The experiments undertaken, however, were not such that sweeping negative conclusions might be drawn in regard to the action of lecithin and cholesterin under different conditions. If lecithin and cholesterin are of importance in this connection, however, their action is very complex and is possibly dependent upon or influenced by other substances present in the serum. Before proceeding with further investigations along this line it appeared wise, therefore, to study other substances in the serum and to study the antihemolytic property of serum after various methods of treatment calculated to throw light on these points.

Many observations appear in the literature that are suggestive as to the substances other than the lipoids of serum which may be of importance in relation to its antihemolytic property. Joannovics and Pick [3] stated that hemolysis by oleic acid is inhibited by organ albumen. Fenyvessy [4] made the observation that hemolysis by soap solution is inhibited by albumen, but expressed the belief that some of this action is due to the calcium present. Liebermann [5] observed that serum albumen protected against hemolysis by soap and that similar protection is afforded by calcium solution. Lamar,[6] also, attributed to the action of serum proteins the protection afforded by serum against hemolysis of red blood cells and lysis of pneumococci by sodium oleate, and stated that the hemolytic action of soaps is due partly to their avidity for protein and not wholly to their ability to dissolve lipoids. Lamar [7] stated, however, that the removal of calcium from the serum did not alter the protective power exhibited by serum against hemolysis by soaps, but that the addition of boric acid to serum did diminish this protective power. Bayer [8] observed that the protective power of serum against hemolysis by bile salts was destroyed by digesting the serum with pepsin or trypsin, and suggested that the protective power of normal serum against this hemolysin was due to the serum albumen bodies. On the other hand, Sellards,[9] while studying the protective power of serum against hemolysis by bile and bile salts, found that precipitation of the proteins by heat did not affect this inhibitory property of the serum. W. W. Ford [10] reported that hemolysis by phallin, the hemolytic principle of a poisonous

mushroom (*Amanita phalloides*), was inhibited not only by blood serum but also by raw and boiled milk. The work of the observers mentioned, although not in entire accord, suggests therefore both albumen and calcium as possible factors in the protective power against hemolysis exhibited by blood serum. Evidence has also been presented to show that boric acid added to serum lowers its protective power against hemolysis by sodium oleate.

The experiments to be reported in this paper were undertaken to determine, if possible, what influence albumen, calcium and boric acid have on hemolysis by sodium oleate and saponin, and what relation these substances may have to the antihemolytic property of serum against these hemolytic agents. Similar experiments were carried out with dextrose and soluble starch.

To study these points quantitative measurements were made of the protective power of albumen, of calcium solution, and of the carbohydrate solutions against hemolysis by sodium oleate and saponin. Similar quantitative measurements were made of water and salt solution extracts of the dried residue of serum, of serum after boiling, after dialyzing, after digestion with trypsin, pepsin, and diastase, and after the addition of boric acid. The technique of making quantitative measurements of the protective power against hemolysis was the same as that reported in earlier papers on the protective power of human serum against hemolysis by sodium oleate,[1] and on studies of the possible rôle of lecithin and cholesterin in hemolytic processes.[2] A number of tubes, each containing 2 c. c. of a different dilution of hemolytic agent were set up in series and to each tube 0.25 c. c. of the material to be tested for protective power against hemolysis was added. This was heated at 37° C. in the water-bath for one-half hour, cooled for one-half hour, and then 0.25 c. c. of a 0.75 per cent suspension of washed guinea-pig cells was added as indicator. This preparation was incubated at 37° C. in the water-bath for two hours, allowed to sediment in the ice-box for one hour, after which a reading of hemolysis was made. The details of the technique are given in the earlier papers and will not be repeated here. In those titrations of protective power of serum against hemolysis, as in the ones in this paper, the tubes carried dilutions of sodium oleate from 1-45,000 to 1-100,000 in steps of 5000, and dilutions of saponin from 1-18,000 to 1-34,000 in steps of 2000. In such a series, 0.25 c. c. or normal serum diluted 1-20 protected against hemolysis by sodium oleate up to strengths of 1-50,000 and 1-55,000; and protected against hemolysis by saponin up to strengths of 1-18,000 and 1-20,000.

I. Studies with Proteins

The properties of proteins in relation to hemolysis by sodium oleate and saponin were studied by several methods.

(a) The protective power of serum against hemolysis was determined after

(1) Boiling and

(2) Boiling and filtering.

(b) The protective power of serum against hemolysis was determined after digestion with

(1) Pepsin.

(2) Trypsin.

(c) The protective power against hemolysis of crystallized egg albumen dissolved in salt solution was determined.

(d) The protective power of dialyzed serum was determined both before and after the precipitated globulins had been removed by filtration.

(a) For the studies of boiled serum, normal serum was prepared for titration in the manner already detailed in a previous paper. The blood was taken by veni-puncture into a clean, dry syringe, expelled into a clean, dry test-tube, allowed to clot, and within one hour after the veni-puncture the serum was removed from the clot and immediately inactivated in the water-bath at 56° C. for one hour. This was kept in the ice-box till just before use—always within twenty-four hours from the time of veni-puncture—and then diluted 1-20 with normal salt solution. The serum thus prepared was divided into four portions. One portion was boiled vigorously for five minutes; another portion was boiled the same way and filtered through two layers of filter paper and kieselguhr; and another portion was similarly filtered but not boiled. With these three specimens and with the fourth which was neither filtered nor boiled titrations for protective power against hemolysis by sodium oleate and saponin were carried out. They resulted as follows:

Sodium Oleate

Normal serum—partial hemolysis up to and including dilution 1-50,000.
Boiled serum—partial hemolysis up to and including dilution 1-65,000.
Boiled serum, filtered—partial hemolysis up to and including dilution 1-65,000.
Filtered serum—partial hemolysis up to and including dilution 1-50,000.

Saponin

Normal serum—partial hemolysis up to and including dilution 1-20,000.
Boiled serum—partial hemolysis up to and including dilution 1-28,000.
Boiled serum, filtered—partial hemolysis up to and including dilution 1-28,000.
Filtered serum—partial hemolysis up to and including dilution 1-20,000.

These results show that the boiling of serum lowers its protective power against hemolysis by sodium oleate and saponin. This diminution is not increased by filtration of the boiled serum.

(b) For studies of the action of ferments on the protective power of serum against hemolysis, pepsin was added to 10 c. c. of normal serum prepared as above and trypsin added to another 10 c. c. portion. These preparations were incubated at 37° C. over night, then filtered through kieselguhr till clear, and inactivated at 56° C. in the water-bath for one hour. Normal serum not treated with ferments was similarly incubated, filtered and inactivated at the same time. Titrations with these preparations and of normal untreated serum for

protective power against hemolysis by sodium oleate and saponin resulted as follows:

SODIUM OLEATE

Normal serum—partial hemolysis up to and including dilution 1-55,000.

Serum digested with trypsin—complete hemolysis up to and including dilution 1-90,000 plus.

(This hemolysis was complete at the end of an hour's incubation. No doubt it would have gone far above 1-90,000 had the dilutions been carried further.)

Serum digested with pepsin—partial hemolysis up to and including dilution 1-90,000.

Serum incubated and filtered—partial hemolysis up to and including dilution 1-55,000.

SAPONIN

Normal serum—partial hemolysis up to and including dilution 1-18,000.

Serum digested with trypsin—complete hemolysis up to and including dilution 1-30,000 plus.

(This hemolysis was complete at the end of half an hour's incubation. The hemolysis would have been seen in dilutions much higher than 1-30,000 if the titration had been carried further.)

Serum digested with pepsin—partial hemolysis up to and including dilution 1-26,000.

Serum incubated and filtered—partial hemolysis up to and including dilution 1-18,000.

The results obtained in these experiments show that, if serum is digested with trypsin or pepsin, the protective power againt hemolysis by sodium oleate and saponin is markedly diminished. When serum is digested with trypsin the reduction in protective power is so severe that it almost amounts to a complete destruction of this property.

The reduction in protective power shown in these experiments might be due to several factors. That it is due to the action of the ferments on the serum is shown by control experiments carried out to establish this point:

(1) Serum was treated with ferments that had been killed and under these conditions there was no diminution in its antihemolytic power.

(2) Ferments were added to salt solution, the salt solution was incubated, inactivated, and filtered as was done with serum. The preparation showed no hemolytic activity.

(3) Serum treated with live ferments and then inactivated showed no hemolytic properties.

(c) To study the influence of crystallized egg albumen on hemolysis by sodium oleate and saponin, albumen crystals prepared by Dr. V. R. Mason according to the method of Hopkins and Pinkus[11] were used. Of these egg albumen crystals, 70 milligrams were dissolved in 10 c. c. of distilled water. This was divided into two 5 c. c. portions and one portion filtered five times through filter paper till water clear, to remove any trace of globulin present. To each of these 5 c. c. portions 45 milligrams of sodium chloride were added, resulting in a solution of albumen in 0.9 per cent salt solution containing about twice as much albumen as normal serum after it has been diluted 1-20 as used in our titrations for protective power against hemolysis. The solution of crystallized egg albumen was used in the titration as serum diluted 1-20, that is, 0.25 c. c. was put in each tube of the series, so that in each tube there was twice as much egg albumen as there was serum albumen in each tube when serum was used. Titrations of the protective power against hemolysis were made with each of the solutions of crystallized egg albumen prepared as above and these compared with a hemolytic series set up with guinea-pig corpuscles unprotected by serum or other substance. In this last hemolytic series the usual volume, 2.5 c. c. in each tube of the hemolytic series, was made up by the addition of 0.25 c. c. of sterile normal salt solution instead of 0.25 c. c. of serum diluted 1-20. The results obtained were as follows:

SODIUM OLEATE

Unprotected guinea-pig corpuscles—partial hemolysis up to and including dilution 1-160,000.

Albumen solution, filtered—complete hemolysis up to and including dilution 1-170,000 plus.

Albumen solution, unfiltered—complete hemolysis up to and including dilution 1-170,000 plus.

(In these last two titrations hemolysis would probably have gone further but the series was not carried to dilutions greater than 1-170,000.)

SAPONIN

Unprotected guinea-pig corpuscles—partial hemolysis up to and including dilution 1-80,000.

Albumen solution, filtered—partial hemolysis up to and including dilution 1-80,000.

Albumen solution, unfiltered—partial hemolysis up to and including dilution 1-80,000.

These experiments demonstrate that crystallized egg albumen dissolved in normal salt solution, used in amounts twice as great as serum albumen when serum is used in a titration for protective power against hemolysis, does not afford protection against hemolysis by sodium oleate and saponin. On the contrary, the presence of egg albumen seems to increase the hemolytic power of sodium oleate.

(d) In studying the relation of the electrolytes of serum to hemolysis by sodium oleate and saponin, some normal serum was dialyzed and this serum was tested for protective power againt hemolysis. Some of the dialyzed serum was filtered to remove the precipitated globulins and this globulin-free, or at least globulin-poor, serum also was tested for protective power against hemolysis. This experiment will be given in detail below; but it is interesting to observe here that the diminution of protective power which is found to result from the dialysis of serum is increased a little, but only to a negligible degree, by removal of the precipitated globulins by filtration.

II. STUDIES WITH CALCIUM

To determine, if possible, what rôle calcium salts play in the protective power exhibited by serum against hemolysis by sodium oleate and saponin, several different experiments were carried out.

(a) Serum was treated with potassium oxalate and the protective power of the serum thus treated was tested.

(b) Calcium chloride was added to serum and this serum was titrated for protective power against hemolysis.

(c) Solutions of calcium chloride in normal salt solution were tested for protective power against hemolysis.

(a) To determine the effect on protective power against hemolysis when any available calcium in the serum is rendered inert as far as possible by treating it with potassium oxalate, 500 milligrams of potassium oxalate crystals were added to 0.5 c. c. of serum. This was allowed to stand for thirty minutes and was then diluted to 10 c. c. with sterile normal salt solution and kept in the incubator at 37° C. over night. The next morning just before use the crystals remaining were thoroughly sedimented by centrifugation. Titrations carried out with this oxalated serum diluted 1-20 resulted as follows:

Sodium Oleate

Normal serum—partial hemolysis up to and including dilution 1-55,000.
Oxalated serum—partial hemolysis up to and including dilution 1-65,000.

Saponin

Normal serum—partial hemolysis up to and including dilution 1-20,000.
Oxalated serum—partial hemolysis up to and including dilution 1-20,000.

These experiments show that, if serum is oxalated, its protective power against hemolysis by sodium oleate is diminished slightly. They also show that this treatment does not lower the protective power against hemolysis by saponin.

(b) The experiment detailed above shows that oxalated serum has lost some of its protective power aginst hemolysis by sodium oleate as compared with normal serum. It seemed worth while, therefore, to add calcium chloride to serum to see if thereby its protective power against hemolysis would be increased. One specimen of serum was diluted 1-40 with a solution of calcium chloride in normal salt solution. In this solution there were 62 milligrams of calcium chloride per 100 c. c. of normal salt solution. The preparation resulting was serum diluted 1-40 (or one-half the strength as ordinarily used) in the presence of a great excess of calcium chloride, as compared with a similar quantity of whole normal blood. Another portion of the same serum was diluted 1-40 with sterile normal salt solution. Titrations carried out with these preparations and with the same serum diluted 1-20 as in the ordinary technique resulted as follows:

Sodium Oleate

Normal serum (1-20)—partial hemolysis up to and including dilution 1-55,000.
Normal serum (1-40)—partial hemolysis up to and including dilution 1-85,000.
Normal serum (diluted 1-40 with normal salt solution containing 62 milligrams of calcium chloride per 100 c. c.)—partial hemolysis up to and including dilution 1-70,000.

Saponin

Normal serum (1-20)—partial hemolysis up to and including dilution 1-20,000.
Normal serum (1-40)—partial hemolysis up to and including dilution 1-24,000.
Normal serum (diluted 1-40 with normal salt solution containing 62 milligrams of calcium chloride per 100 c. c.)—partial hemolysis up to and including dilution 1-24,000.

These experiments show that calcium chloride, when added to serum in very small amounts, but relatively in great excess as compared with that present in normal blood serum when diluted 1-20 for use, is capable of increasing the protective power of serum slightly against hemolysis by sodium oleate. This same procedure does not increase the protective power of serum against hemolysis by saponin.

(c) In order to study further the possible rôle of calcium in the blood in relation to the protective power against hemolysis exhibited by serum, 62 milligrams of calcium chloride were dissolved in 100 c. c. of normal salt solution and this preparation was tested for protective power against hemolysis. It was used just as normal serum diluted 1-20, *i. e.,* 0.25 c. c. of the solution of calcium chloride in normal salt solution was added to each tube of the hemolytic series. Since the calcium solution contained much more calcium than serum, and was used without dilution, there were many more times as much calcium in each tube of the hemolytic series as when serum is used in the ordinary titration. The readings made were as follows:

Sodium Oleate

Normal serum—partial hemolysis up to and including dilution 1-55,000.
Calcium in salt—complete hemolysis up to and including dilution 1-90,000.
(The hemolysis would probably have gone higher than 1-90,000, but the titration was not carried further.)

Saponin

Normal serum—partial hemolysis up to and including dilution 1-20,000.
Calcium in salt—complete hemolysis up to and including dilution 1-30,000.
(The hemolysis up to 1-30,000 was complete almost immediately and would probably have gone further had the titration been carried on to greater dilutions.)

These experiments show that calcium chloride in salt solution, present in much greater concentration than when serum is used, affords little, if any, protection against hemolysis by sodium oleate. The calcium chloride solution does, however, slow the reaction somewhat with sodium oleate, for at the end of 1½ hours' incubation hemolysis had proceeded only to dilution 1-70,000, whereas, in all the titrations set up with unprotected guinea-pig corpuscles previously recorded, hemolysis at that time had always proceeded to dilutions above 1-100,000 and sometimes as high as dilutions of 1-150,000. Such a calcium chloride solution does not protect against hemolysis by saponin.

III. Studies with Carbohydrates

There is nothing to suggest sugar as a substance in the serum of importance in relation to protective power against hemolysis, and we have no reason to expect that sugar can inactivate a hemolytic agent. Evidence has been presented, however, to show that a medium containing sugar will preserve red blood cells better than a similar solution without sugar. Carbohydrates might, therefore, be of great importance in hemolysis, if not as a substance neutralizing the hemolytic agent, at least, perhaps, rendering the red blood

cells less subject to its disintegrating influence. Furthermore, carbohydrates, as important constituents of normal blood, are worthy of some study in relation to their protective power against hemolysis. This point was investigated by several methods:

(a) Quantitative measurements were made of the antihemolytic power of a solution of dextrose and of soluble starch in normal salt solution.

(b) Dextrose and soluble starch were added to serum, and quantitative measurements were made of the antihemolytic power of serum thus treated.

(c) Serum after digestion with diastase was tested for protective power against hemolysis.

(a) One hundred milligrams of pure dextrose were dissolved in 100 c. c. of sterile normal salt solution. The salt solution then contained the same concentration of sugar as normal blood. It was further diluted 1-5, and 0.25 c. c. of this preparation was put in each tube of the hemolytic series so that the sugar was four times as strong as in the serum diluted 1-20 used in the ordinary test. A similar preparation with equal quantities of soluble starch * was prepared and used in the same way. The titrations carried out resulted as follows:

Sodium Oleate

Normal serum—partial hemolysis up to and including dilution 1-55,000.
Dextrose in salt solution—complete hemolysis up to and including dilution 1-90,000.
Soluble starch in salt solution—complete hemolysis up to and including dilution 1-90,000.
(The hemolysis in the last two tests was complete after half an hour's incubation. It would have gone far above 1-90,000 had the dilutions been carried further.)

Saponin

Normal serum—partial hemolysis up to and including dilution 1-18,000,
Dextrose in salt solution—complete hemolysis up to and including dilution 1-30,000.
Soluble starch in salt solution—complete hemolysis up to and including dilution 1-30,000.
(The hemolysis in the last two titrations was complete after half an hour's incubation. It would have gone far above 1-30,000 had the dilutions been carried further.)

These experiments show that neither dextrose nor soluble starch dissolved in salt solution protects against hemolysis by sodium oleate or saponin.

(b) Normal serum was inactivated as usual and 10 milligrams of dextrose were added to one 5 c. c. portion and 10 milligrams of soluble starch to another. The carbohydrates dissolved readily in the serum. These preparations were kept in the ice-box over night. Dilution of 1-20 was carried out as usual just before use and titration for any protective power of these sera and a normal serum prepared in the usual way yielded the following results:

Sodium Oleate

Normal serum—partial hemolysis up to and including dilution 1-55,000.
Normal serum plus soluble starch—partial hemolysis up to and including dilution 1-50,000.
Normal serum plus soluble starch—partial hemolysis up to and including dilution 1-55,000.

Saponin

Normal serum—partial hemolysis up to and including dilution 1-18,000.
Normal serum plus dextrose—partial hemolysis up to and including dilution 1-20.000.
Normal serum plus soluble starch—partial hemolysis up to and including dilution 1-20,000.

These experiments show that serum to which dextrose or soluble starch has been added, even in relatively large quantities (200 milligrams of carbohydrate per 100 c. c. serum) shows no appreciable alteration in protective power against hemolysis by sodium oleate and saponin. The titrations recorded are within the limits of normal with the technique used.

(c) Diastase was added to 10 c. c. of normal serum and this solution was incubated for eighteen hours. It was then filtered twice through kieselguhr and inactivated in the waterbath at 56° C. for one hour. Some serum from the same source not treated with diastase was similarly incubated, filtered, and inactivated. Dilution of 1-20 was carried out as usual and titration for the protective power of these sera and of a normal serum prepared in the usual way yielded the following results:

Sodium Oleate

Normal serum—partial hemolysis up to and including dilution 1-55,000.
Normal serum (incubated and filtered but not treated with any ferment)—partial hemolysis up to and including dilution 1-55,000.
Normal serum after incubation with diastase—complete hemolysis up to and including dilution 1-90,000 (the hemolysis in dilutions as far as carried 1-90,000 was complete after one hour's incubation).

Saponin

Normal serum—partial hemolysis up to and including dilution 1-18.000.
Normal serum (incubated and filtered but not treated with any ferment)—partial hemolysis up to and including dilution 1-18,000.
Normal serum after incubation with diastase—complete hemolysis up to and including dilution 1-30,000 (the hemolysis was complete in all the dilutions set up after one-half hour's incubation).

These experiments demonstrate that, when serum is digested with diastase, the protective power against hemolysis by sodium oleate and saponin is markedly diminished, if not completely destroyed. This diminution in protective power is due to the action of the ferment on the serum as shown by control experiments carried out with the diastase as with trypsin and pepsin already detailed:

(1) Serum treated with inactivated ferment did not show a diminution in antihemolytic property.

(2) Salt solution treated with diastase showed no hemolytic activity.

* The soluble starch used was obtained from the Department of Physiological Chemistry of The Johns Hopkins Medical School through the kindness of Professor Walter Jones. It was a specimen made from compressed yeast in an attempt to get yeast nucleic acid by the method of Clark and Schriver.

(3) Serum treated with live ferments and then inactivated showed no hemolytic activity.

IV. Miscellaneous Studies

In addition to studies with lecithin and cholesterin[2] and with proteins, calcium, and carbohydrates, certain other investigations were carried out in a search for the substance or substances in the serum responsible for the protective power exhibited by it against hemolysis by sodium oleate and saponin.

(a) The dried residue of serum taken up in distilled water and in sterile normal salt solution was tested for protective power against hemolysis.

(b) The protective power of serum after dialysis was measured quantitatively.

(c) Quantitative measurements of the protective power of serum after the addition of boric acid were made; and of boric acid dissolved in normal salt solution.

(a) To determine whether any protective substance against hemolysis by sodium oleate and saponin could be demonstrated in the dried residue of serum, several methods of drying the serum were tried, and that found most satisfactory and apparently least likely to introduce unknown factors was drying in the air. Serum in accurately measured 2 c. c. portions was put on chemically clean, sterile watch crystals and these, protected from dust as much as possible, were dried for twenty-four hours in front of an electric fan. This dried residue was pulverized very thoroughly and that derived from each 2 c. c. portion of serum was put in a separate test tube. Ten cubic centimeters of sterile distilled water were added to two of the tubes containing the pulverized residue from 2 c. c. serum, and ten cubic centimeters of sterile normal salt solution were added to two others. One of these to which water and one to which normal salt solution had been added, were boiled gently for five minutes. This resulted in four preparations:

(1) Dried residue of serum in cold water.

(2) Dried residue of serum boiled in water.

(3) Dried residue of serum in cold normal salt solution.

(4) Dried residue of serum boiled in normal salt solution.

When the water or salt solution was added to the powdered serum residue and shaken, the preparations took on a cloudy, brownish appearance. They were put in the incubator at 37° C. over night and when looked at in the morning no sedimentation had occurred in the tubes that had been boiled and practically none in the others. Attempts made to clear the solutions by centrifugation were unsuccessful. A portion of each preparation filtered twice through ordinary filter paper did not become clear. Accordingly, titrations for any protective power against hemolysis were caried out with these suspensions (rather than extracts) of the whole dried residue of serum, using them exactly as serum diluted 1-20; *i. e.,* 0.25 c. c. was put in each tube of the hemolytic series. In these preparations the serum components were present in four times the strength that they were in normal serum as used in a titration. Constant quantitative results could not be obtained on repetition of these experiments, but the gross results were such that definite conclusions might be drawn. For example the titrations of one day resulted as follows:

Sodium Oleate

Serum residue in cold water—no hemolysis in dilution 1-50,000.
Serum residue boiled in water—no hemolysis in dilution 1-50,000.
Serum residue in cold normal salt solution—hemolysis in dilution 1-50,000, but not in 1-55,000.
Serum residue boiled in normal salt solution—hemolysis in dilution 1-50,000, but not in dilution 1-55,000.

Saponin

Serum residue in cold water—no hemolysis in dilution 1-18,000.
Serum residue boiled in water—no hemolysis in dilution 1-18,000.
Serum residue in cold normal salt solution—no hemolysis in dilution 1-18,000.
Serum residue boiled in normal salt solution—no hemolysis in dilution 1-18,000.

Other experiments carried out in the same way did not duplicate these figures exactly, but demonstrated, as do these, that such a preparation of the dried residue of serum has marked protective power against hemolysis by sodium oleate and saponin.

(b) To determine what influence the removal of electrolytes from serum would have on its protective power against hemolysis by sodium oleate and saponin, normal serum was subjected to dialysis and the protective power of such serum measured quantitatively. Normal serum in 5 c. c. quantities was accurately measured into collodion sacks and dialysis carried on in running tap-water for twenty-four hours. The contents of the sack were then put into a chemically clean, sterile container. The dialyzing sack was washed out repeatedly with small amounts of sterile, normal salt solution and these washings added to the other fluid from the sack. Another similar preparation was filtered through kieselguhr till clear to remove the precipitated globulins before the addition of enough salt solution to render them soluble. Each of these preparations was brought up to a total volume of 100 c. c. by the addition of sterile normal salt solution, resulting in a dilution of 1-20 of the 5 c. c. serum originally introduced into the dialyzing sack. This dialyzed serum, one portion filtered before the addition of much salt solution, and another not filtered at all, diluted 1-20 in this manner, was used in a titration for protective power against hemolysis exactly as normal serum diluted 1-20 in an ordinary titration. The readings made were as follows:

Sodium Oleate

Normal serum—partial hemolysis up to and including dilution 1-55,000.
Dialyzed serum—partial hemolysis up to and including dilution 1-80,000.
Dialyzed serum filtered—partial hemolysis up to and including dilution 1-85,000.

Saponin

Normal serum—partial hemolysis up to and including dilution 1-20,000.
Dialyzed serum—partial hemolysis up to and including dilution 1-22,000.
Dialyzed serum filtered—partial hemolysis up to and including dilution 1-30,000.

These experiments show that dialysis of serum lowers the protective power manifested by it against hemolysis by sodium oleate and saponin; and that this diminution of protective power is increased by removal of the precipitated globulins by filtration. In the repetition of the experiments the exact figures given above could not be duplicated, but all were such that the same conclusions were unavoidable. One cannot, however, draw conclusions from them as to the quantitative diminution of protective power against hemolysis after dialysis of serum.

(c) Liebermann and Fenyvessy[4] made the observation that the protective power of serum against soap hemolysis was inhibited by adding boric acid to the serum; and this finding was confirmed by Lamar.[7] In this study it was thought important to test out this property of boric acid in relation to hemolysis by sodium oleate and saponin. Accordingly, quantitative measurements were made of the protective power against hemolysis of:

(a) Normal serum to which boric acid had been added.

(b) A solution of boric acid in salt solution.

(a) Boric acid crystals were added in excess to 0.5 c. c. of serum and this was diluted to 10 c. c. (1-20) with sterile normal salt solution and allowed to stand in the incubator over night. The next morning the excess boric acid crystals were removed by centrifugation. As in the usual titrations 0.25 c. c. of this serum was added to each of the tubes in the hemolytic series. The readings made were as follows:

Sodium Oleate

Normal serum—partial hemolysis up to and including dilution 1-55,000.

Normal serum to which boric acid had been added—partial hemolysis up to and including dilution 1-90,000 (the hemolysis probably would have proceeded further but the titration was not carried to higher dilutions).

Saponin

Normal serum—partial hemolysis up to and including dilution 1-20,000.

Normal serum to which boric acid had been added—partial hemolysis up to and including dilution 1-20,000.

These experiments show that boric acid added to serum markedly reduces the protective power of the serum against hemolysis by sodium oleate, but does not diminish the protective power against hemolysis by saponin.

The control tubes set up with each titration[1] showed that the serum to which boric acid had been added was not in itself hemolytic.

(b) Boric acid crystals in excess were added to sterile salt solution and this was allowed to remain in the incubator over night. Just before use the next morning, the excess boric acid crystals were removed by centrifugation and 0.25 c. c. of the clear solution was added to each tube of the hemolytic series. At the end of one and one-half hour's incubation the hemolysis was complete as far as the titrations were carried; *i. e.,* 1-90,000 for sodium oleate, and 1-30,000 for saponin. This experiment indicates that boric acid in itself has no protective power against hemolysis. It further showed that boric acid has little if any power to cause hemolysis, for under the conditions of the experiment the addition of even a weak hemolytic agent, instead of the salt solution to which boric acid had been added, would have caused complete hemolysis almost immediately in all dilutions prepared, instead of after one and one-half hour's incubation.

Summary of Findings

(I) The boiling of serum lowered slightly its protective power against hemolysis by sodium oleate and saponin. The protective power of serum was very markedly diminished also by digestion of the serum by trypsin and pepsin. Egg albumen did not exhibit protective power against hemolysis by these agents.

(II) Serum treated with potassium oxalate showed a slight diminution in protective power against hemolysis by sodium oleate, but no reduction in protection against hemolysis by saponin. Calcium chloride added to serum increased slightly the protective power against hemolysis by sodium oleate, but not by saponin. Calcium chloride in salt solution exhibited no protective power against hemolysis by sodium oleate or saponin, but did apparently slow the progress of the hemolytic process with sodium oleate.

(III) Dextrose, or soluble starch dissolved in salt solution had no protective power against hemolysis by sodium oleate or saponin. Serum digested with diastase, however, showed a marked reduction in protective power against both sodium oleate and saponin.

(IV) (a) The dried residue of serum when suspended in distilled water or normal salt solution exhibited marked protective power against hemolysis by both sodium oleate and saponin.

(b) Serum when dialyzed exhibited a marked diminution in protective power against hemolysis by both sodium oleate and saponin. When the dialyzed serum was filtered to remove the precipitated globulins, the diminution in protective power was increased.

(c) Serum treated with boric acid showed a diminution in protective power against hemolysis by sodium oleate, but no diminution in protection against saponin hemolysis. Boric acid in salt solution had no protective power against either sodium oleate or saponin hemolysis.

It is very difficult to draw conclusions from the experiments discussed above. The fact that when serum is boiled, thereby coagulating the albumen, the protective power against hemolysis by both hemolytic agents is diminished indicates that this property of the serum is in some way dependent upon the albumen present. The diminution in protective power resulting from digestion of the serum by pepsin and trypsin lends further support to this supposition. If this is correct, however, it is some property peculiar to albumen found in serum or perhaps closely allied proteins, or a property dependent upon the physical state in which the albumen is held in the serum, since crystallized egg albumen dissolved in normal salt solution exhibits no protective power against hemolysis by the hemolytic agents used. The fact that crystallized albu-

men has no protective power also suggests that the protective power of the serum may be due to the globulin fraction. When the globulins of the serum were precipitated by dialysis and removed by filtration, it was seen that there was a slight diminution in protective power as compared with that afforded by serum dialyzed but not filtered. This would indicate that the globulin fraction is of some importance, but the difference between dialyzed serum from which the globulin had been largely removed and dialyzed serum from which the globulin had not been removed was so slight that no conclusions may be drawn from it, except that probably the globulin had little if anything to do with the inhibition of hemolysis by sodium oleate and saponin. It must also be kept in mind that the diminution in protective power of serum after boiling, after digestion, and after dialysis and filtration is possibly due in part at least to changes in the serum other than those produced in the protein fraction. So, although these experiments are strongly suggestive that the albumen of the serum, or at least the physical state in which it is held, is an important factor in the inhibition by serum of hemolysis by sodium oleate and saponin, they are not conclusive.

The experiments with calcium salts indicated definitely that calcium affords no protection against saponin hemolysis. The protection exhibited by calcium salts against hemolysis by sodium oleate was very slight, even though the calcium salts were present in more than twenty times the strength normally occurring in the serum as used. This can probably be explained by the fact that insoluble calcium oleate was formed from part of the sodium oleate present. These results seem to justify the conclusion that the calcium in serum is not responsible for the protective power manifested by it against hemolysis by sodium oleate and saponin, and that, if it plays any rôle at all in this protective power, it is indirect.

The finding that serum digested with diastase shows marked diminution in protective power against both hemolytic agents used, suggested the possibility that the carbohydrates of the serum were important factors in the phenomena under consideration. But when it was found that neither dextrose nor soluble starch exhibited protective power against hemolysis by these hemolysins, and that none of these substances added to serum increased its antihemolytic power, the conclusion was unavoidable that the diminution in protective power was not dependent upon changes in the carbohydrates of the serum by the action of diastase. It seems more probable that the diminution in protective power was due to other changes taking place in the serum as a result of the diastatic action.

The protective power of the dried residue of serum suspended in normal salt solution and in distilled water showed that this property of the serum is not destroyed by drying. The diminution of the protective power of serum resulting from removal of the electrolytes by dialysis indicates that these are of importance, but the negative findings, in direct experiments with calcium salts alone, do not support this hypothesis, at least as far as calcium salts are concerned. Again, it is probable that it is not merely the removal of the electrolytes but other changes in the serum incident to this procedure which are responsible for the resulting diminution in protective power.

It appears almost certain that the protection by serum against hemolytic agents is a colloidal phenomenon and that whatever the substance in the serum may be which exerts this action, the physical state in which it is held in the serum is important. It is possible, therefore, that many of the procedures carried out in these experiments which altered the colloidal state of the serum were, by reason of this action, responsible for the changes in protective value of the serum, rather than any effect they had on any one particular substance in the serum. Boiling and drying serum, the changes in reaction incident to digestion of serum by trypsin, pepsin and diastase, and removal of electrolytes, so altered the physical properties of the serum that if the physical state in which the protective substance is held in the serum is important, no further explanation for the reduction in protective power need be sought. Indeed the reduction in protective power of serum resulting from such widely different procedures, leads to the conclusion that the physical state of the serum is important and that the protection afforded by serum against hemolysis by certain hemolytic agents is a very complex process.

Conclusions

(1) Treatment of serum which alters the physical state or removes all or a part of the proteins diminishes the protective power of the serum against hemolysis by sodium oleate and saponin.

(2) Calcium alone is not the substance in serum upon which the protective power of the serum against hemolysis by sodium oleate and saponin depends.

(3) Dextrose or soluble starch have no power to protect against hemolysis by sodium oleate and saponin *in vitro* and probably, therefore, carbohydrates exert no such influence in the blood.

(4) Boric acid added to serum diminishes the protective power against hemolysis by sodium oleate, but does not influence the protection against hemolysis by saponin.

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THE NUCLEUS IN THE HUMAN RESTIFORM BODY[1]

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Introduction

While studying the human brain I came across a small nucleus in the restiform body, which, so far as I can learn, has not been described in the literature. It occurred to me that this nucleus was probably constant in the human restiform body, and as a result, I undertook the examination of quite a number of human brains. I was doubtful at first whether the nucleus, which I am about to describe, could always be demonstrated, but thorough examination revealed its presence in the restiform body of all the human brains that I examined. The results of these studies follow.

Report of Cases

All brains were fixed in formaldehyde solution and cut transversely in serial sections, each 25 microns thick, from the medulla oblongata and pons; the sections were then stained by the Nissl method and microscopic studies made. The observations have been tabulated and figured as follows:

In addition to the tabulated cases, the brains of one adult and one fetus, stained by the Nissl method, and those of three adults, stained by the Weigert method, were examined. In every one of these cases there was found in the restiform body a small nucleus, occupying a position similar to that described in the previous cases. None shows the hilum of the first case, although in some there is evidence of a slight curvature in transverse sections, suggesting a very incomplete hilum in the nucleus. The long axis of the nucleus, when it appears as an elongated mass, in transverse sections, always runs in a dorso-medial-ventro-lateral direction. In the Weigert preparations, a dense mass of nerve fibers is seen streaming to and from the hilum and also surrounding it. However, there is no evidence as to the origin of these nerve fibers.

The Weigert studies of the cells found in the nucleus are omitted in this paper, since this method of study does not lend itself to our present purpose. The fetus stained by the Nissl method is not described in detail here, because some of the

Case	Upper and lower levels	Position in restiform body	Development of nucleus	Form of nucleus	Longitudinal diameter	Size of cells in nucleus	Shape of cells in nucleus	Hilum
I.	First comes into view at the level where the inferior olive is marked and the superior vago-glosso-pharyngeal roots are still seen. Disappears where the inferior portion of the accessory auditory nucleus appears.	Appears as a small mass of gray matter in the dorso-medial part of the restiform body (see Fig. 1).	Fully developed.	⊃-shaped (see Fig. 5).	Distance between inferior and superior poles, 1.3 mm.	11.1–22.2 μ..	Round or fusiform.	Pronounced.
II.	Same	Same	Poor..........	Round or elliptical. (Fig. 6.)	0.6 mm.......	7.8–18.5 μ...	Same........	Absent or imperfect.
III.	Same	Same	Poor..........	Elongated with constriction in middle. (Fig. 7.)	0.9 mm.......	7.4–22.2 μ...	Same........	......
IV.	Same	Same	Traces only...	Round. (Fig. 8.)	0.6 mm.......	11.1–18.5 μ..	Same........	Absent.
V.	Same	Same	Traces only...	Round. (Fig. 9.)	0.5 mm... ...	11.1–25.9 μ..	Same........	Absent.
VI.	Same	Same	Poor..........	Round. (Fig. 10.)	0.6 mm.......	11.1–22.2 μ..	Same........	Absent.
VII.	Same	Same	Not advanced.	Round. (Fig. 11.)	0.8 mm.......	11.1–25.9 μ..	Same........	Incomplete.
VIII.	Same	Same	Not advanced.	Round. (Fig. 12.)	0.6 mm.......	11.1–18.5 μ..	Same........	Incomplete.

[1] Read before the thirty-first annual meeting of the Okayama Medical Association and the nineteenth annual meeting of the Japanese Neurological Association.

Fig. 1

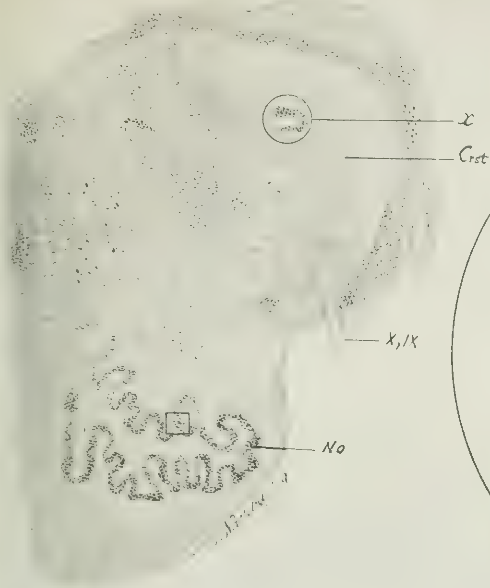

Fig. 2

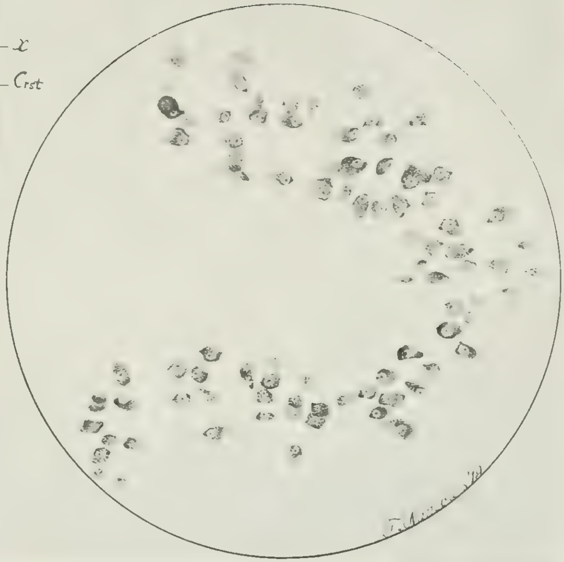

Fig. 3

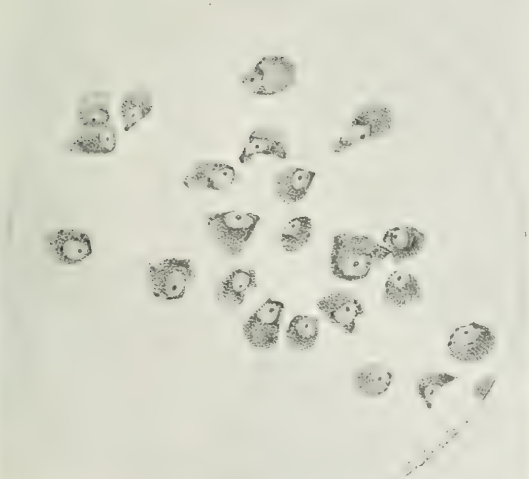

Fig. 4

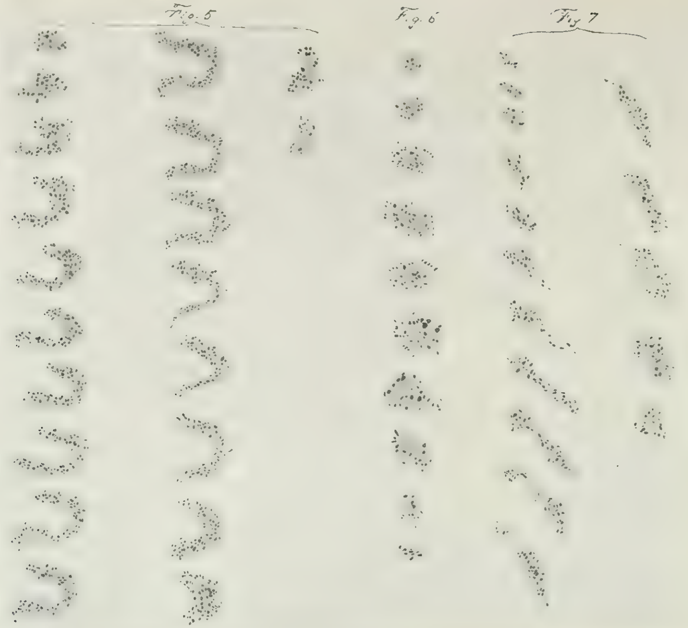
Fig. 5
Fig. 6
Fig 7

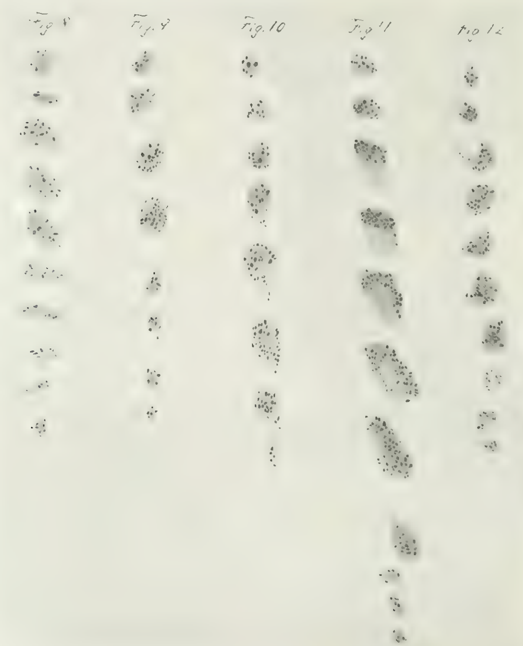
Fig. 10

sections were not well stained and others were not cut in good series.

Discussion

Thirteen human brains in all were examined. The nuclei in the restiform bodies in eight of them have been described in the foregoing table. With the exception of the eighth case, which is that of a fetus 44.5 cm. long, they included brains of adults of both sexes. The remaining five cases were those of four adults and one fetus.

In all of these cases it was possible to demonstrate a peculiar nucleus, which, in transverse sections, appears in the dorso-medial part of the restiform body and is present in both embryonic and adult specimens. The inferior border of this nucleus is seen in the restiform body, about the level of the posterior vago-glosso-pharyngeal roots, and its superior border disappears just as or after the accessory auditory nucleus appears. Hence its longitudinal diameter is greater than the transverse, and averages in length about 0.5 to 1.3 mm.

The hilum of the nucleus is directed mesially, when it is well developed, as in Case I. In most cases, however, it is either absent or very indefinite, and the nucleus itself is also in an undeveloped stage, appearing in transverse sections as a small round or elliptical mass.

The following are the characteristics common to all the cases:

(1) The situation of the nucleus in the restiform body is the same in all cases.

(2) In transverse sections, when the nucleus appears elongated, the long axis runs always in a dorso-medial-ventro-lateral direction.

(3) When the nucleus appears elongated in transverse sections, it is curved and shows an incomplete hilum.

(4) The type of nerve cells in the nucleus is the same for all cases.

The type of cell found in this nucleus is similar to that of the inferior olive. The cells appear round or somewhat elliptical, and in size vary from 7.4 to 25.9 microns. The cells are uniformly scattered in the gray matter. Sometimes, however, they are more particularly collected in the periphery of the gray matter, or, more rarely, a very few nerve cells may exist outside, and not very close to, the gray matter.

The brains of certain other mammals, for instance, the monkey, dog, cat, rabbit and guinea-pig were used for similar studies, but in these thorough examination failed to reveal any such nucleus in the restiform body. Hence, it is possible that this nucleus that I have described is peculiar to the human species.

The physiology of the nucleus and its relation to the other parts of the brain are entirely unknown. It is interesting to note that the nucleus is found only in that species in which the inferior olive reaches its highest development, but not in the animals which show but a slight development of the inferior olive. This suggests that the nucleus may be a portion of the inferior olive, which has found its way into the restiform body. This hypothesis is suggested by the following findings:

(1) The nucleus presents itself in the restiform body when the inferior olive shows its greatest development.

(2) In some cases the nucleus has a hilum which bears some resemblance to a part of the inferior olive.

(3) The nerve cells of the nucleus bear a striking resemblance in shape to those of the inferior olive (Figs. 3 and 4).

(4) The restiform body and inferior olive are intimately related to each other.

(5) The fact that often only traces of the nucleus are present, and that there is a considerable amount of variation in shape and development in all cases, makes one think that the nucleus may be phylogenetically a young one, first built by man, in whom we find the greatest development of the restiform body and the inferior olive.

Although it must be freely confessed that the above, of course, is nothing but a supposition, nevertheless, judging from the morphological states of the nucleus, it is certainly a logical hypothesis.

Conclusions

(1) There is a nucleus in the restiform body of man.

(2) Tracing the transverse section from below upwards, the nucleus appears where the vago-glosso-pharyngeal root begins to disappear, and disappears where the accessory auditory nucleus begins to appear.

(3) This nucleus is found at the dorso-medial part of the restiform body on transverse sections, and extending in an elongated shape in the medulla oblongata.

(4) The nucleus has no definite shape and shows a considerable amount of variation in growth. On transverse section, the transections are different in form. Some have a hilum tending towards the medial direction, while the others present themselves as long masses extending from the dorso-medial direction to the ventro-lateral. Some of them also show the incomplete hilum-like parts in them, whereas others show only round masses.

(5) The nerve cells of the nucleus are practically identical in shape with those of the inferior olive.

(6) The nucleus is probably a portion of the inferior olive.

It is a great pleasure to acknowledge my indebtedness to Dr. K. Kosaka for his kind advice, and the excellent facilities afforded to me.

EXPLANATION OF FIGURES

Fig. 1.—Transverse section through the middle of the inferior olivary region of the human medulla oblongata (Section No. 169).

No. Inferior olive. *X, IX.* Vago-glosso-pharyngeal roots. *Crst.* Restiform body. *X.* Nucleus in restiform body.

Fig. 2.—Nucleus in the restiform body in Fig. 1, magnified.

Fig. 3.—Nerve-cells of the nucleus in the restiform body in Fig. 1 (high power).

Fig. 4.—Nerve-cells of the inferior olive in Fig. 1, that is, the part in the square (same power).

Figs. 5-12.—Show the shape of the nuclei in the restiform bodies in Cases I to VIII, in transverse sections, arranged systematically from below upwards.

FIG. 5.—Shows the configuration of the nucleus in Case I. The nucleus is composed of nerve-cells and stroma which stain deeply with thionin. Higher up the nerve-cells increase in number, and accordingly various changes occur in the nucleus. The nucleus presents a small round outline at the beginning. Soon, however, it becomes a little bent, its concavity pointing in the dorso-medial direction, and then clearly defined dorsal and ventral spurs develop. As the size of the nucleus increases the ventral spur becomes longer than the dorsal, and its point begins to bend ventrally, so that the nucleus presents an open mouth which might be called the hilum, and appears as a succiform outline (see Figs. 1 and 2). The dorsal spur likewise begins to bend its point dorsally, both spurs finally becoming nearly the same in length and the opening gradually widening. At this stage the whole nucleus assumes the form of the letter U. Still higher up, as the hilum opens wider, the angle between both spurs becomes larger, and with the exception of a slight curve of the ventral spur, the points are no longer bent. The nucleus has gradually increased in size, and shows, in this part, its maximal cross-section, its hilum pointing towards the median raphé. Again, higher up, when the inferior portion of the accessory auditory nucleus appears, the nucleus begins to decrease in size, and shortly afterwards, assuming its original form, it disappears. The nerve-cells of the nucleus, for the most part, tend to become round, but in some instances they are somewhat fusiform (Fig. 3), and approximately of the same size, varying from 11.1 to 22.2 microns. The longitudinal diameter (the distance between the inferior and superior ends) of the nucleus measures about 1.3 mm.

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NOTES ON NEW BOOKS

Injuries and Diseases of the Bones and Joints. By Frederick H. Baetjer and Charles A. Waters. Cloth, $10.00. (*New York, Paul B. Hoeber, 1921.*)

This volume is in many ways distinctive. It is a contribution of the highest order—one in which quality is the keynote throughout—a type which we always look for but seldom find. The usual stereotyped and space-consuming features of most books are omitted. Technique, statistics, theories and the historical side of roentgenology are not considered. Nowhere is there the slightest taint of padding either in writing or illustrations.

It is a purely clinical contribution, dealing only with facts proven beyond question by correlation of the X-ray shadows with the actual anatomical and pathological findings at operation and necropsy. Few roentgenologists, if any, are more qualified by experience, study, and keenness of observation to pass judgment upon the clinical import of roentgenology. It is a big story (covering the entire roentgenological output of The Johns Hopkins Hospital) remarkably condensed and told in a delightfully simple and direct way.

The outstanding features of the work are its thoroughly practical character, and the intimate and accurate views on the problems of medicine and surgery and of the morbid anatomy in each field of study. Fractures, dislocations, joint lesions, bone infections, bone tumors, various abnormalities and dystrophies are considered from every angle of diagnosis. The book is so well balanced that unusual features are not conspicuous. The chapter on bone tumors, however, is particularly impressive. This very complicated and poorly understood subject is reduced to unusual simplicity by the writers' consummate knowledge of the subject from the clinical, pathological and roentgenological aspects.

The book represents the very latest and the most advanced information on the interpretative side of roentgenology. It should be indispensable to all concerned with clinical problems. The illustrations are well selected and clearly reproduced. There is only one regret—that a subject so admirably presented and of such importance should include only a part of clinical roentgenology. It is to be hoped the remainder of the field will shortly be covered by a supplement to this volume.

W. E. D.

Dermatology, The Essentials of Cutaneous Medicine. By Walter J. Highman. (*New York, MacMillan Co., 1921.*)

To the reviewer the appearance at this time of still another text-book on diseases of the skin naturally awakens a sense of critical suspicion. He fortifies himself, however, in the thought that perhaps the author of such a book will bring something new to a field of medicine already progressing vigorously to the point at which respectful attention is being given it. Highman, in his initial effort, has certainly allayed any suspicion of giving us the usual matter-of-fact presentation of the subject.

In the preface, splendidly set forth as it is, he strikes at once at the sore spots which affect this special field, so far as teaching and exposition are concerned. Thus, when he states that the true dermatologist is "an internist who knows the skin" he is stating at once the vital basis on which modern dermatology rests and from which it should be taught. He undoubtedly attempts, thus, to bring back the specialty to the field from which, since 1860, it has strayed perforce through over-enthusiasm and through the unfortunate idea that all skin diseases were of external origin. The schools of Hebra and of Kaposi made such a great stride forward that they left too much behind.

The book is intended for students—being the essentials of cutaneous medicine—but it must certainly appeal also to the busy doctor who wishes to orient himself, briefly, regarding the newer conceptions of the dermatoses. It has a peculiar flavor of originality in so far as an honest attempt is made to clarify our ideas more especially regarding etiology. The author has, of necessity, been compelled to follow in the main a classification of the dermatoses similar to that of the modern treatises; but wherever possible, he has taken the step forward which gives us a saner idea of the causes of the common diseases of the skin. It is, of course, beyond the scope of this review to enumerate this classification.

Despite the necessary brevity of the discussions, Highman has not hesitated to give a relatively protracted exposition of certain diseases. For example, the chapter on urticaria and on the vesicular diseases of the skin certainly makes an eloquent appeal to the critical mind and one must not fail to appreciate that the student in particular needs at times just such a dissertation on a subject so fraught with confusing ideas. The latter chapter (vesicular diseases) is worth alone the writing of the book; for it gives us the most appealing and sensible account of the hitherto confused conceptions of the relationship of the eczemas and the dermatitides. This much should be said in favor of Highman's sensible attitude regarding this point: that even if the passing of the years may prove him to be in error, he has, at least, given the reader and the student a tentative working basis for a simpler and more acceptable comprehension of the one subject which has hitherto made dermatology difficult of understanding, unsatisfying in teaching, and embarrassing in explanation to both the tyro and to the trained worker. Finally, the chapters on syphilis are adequate so far as the size of the book is concerned, very pithy, and certainly quite up to date.

The book, however, is not without some minor faults. The author's experiences in treatment, while much to be commended, are sometimes decidedly debatable, from the standpoint of those of others. In this connection the use of arsenic in psoriasis might be mentioned. In the main, however, his ideas on therapy are excellent and should be helpful—they are brief, practical, and not over-worked; and his prescriptions, happily, are given in the metric system.

He does not mention the possibility of recurrence of the eruption in pityriasis rosea; and unless we mistake, he does not include a description of pellagra.

It is questionable whether the almost complete omission of a brief histological description of the important dermatoses is a wise thing; and further, whether the omission of a brief bibliography has any decided advantage. If the author of a procedure or of a contribution is worth mentioning, it appears to us that some reference to the source would be worth while and would not take up much useful space. Finally, there is fairly frequent evidence of faulty typography—a fault very readily corrected, of course; and there is evidence also of some carelessness in the use of words.

It should be stated, moreover, that the illustrations which number nearly a hundred are of a type of selection and of an excellence which has no peer in any other text-book with which we are acquainted. The volume is of handy size, clearly printed and attractively arranged. And best of all, the author has a literary style which is characterized by such individual ease and clarity of expression, by such original phrasing that one experiences a genuine pleasure in reading the book. Such factors are bound to give a book originality; and having this quality it should certainly win a place as a real contribution to dermatological teaching.

I. R. P.

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THE RADIOGRAPHIC EVIDENCE OF THE INFLUENCE OF COD-LIVER OIL IN RICKETS

By E. A. Park and John Howland

(From the Harriet Lane Home and from the Department of Pediatrics, The Johns Hopkins University)

Cod-liver oil has been employed in medicine for so many years that it is difficult to say when it was actually introduced. Apparently it has been a folk remedy for centuries. During the last hundred years it has been used more or less extensively, particularly with children, for tuberculosis and for almost all diseases associated with wasting. It has also been employed in rickets.

Pharmacological evidence for the beneficial effects of cod-liver oil is slight. According to Cushny [1] it apparently owes its favorable influence chiefly to the fact that it is an easily assimilable form of fat. Meyer and Gottlieb,[2] however, indicate that there may be some virtue in cod-liver oil other than its ready emulsification and easy assimilability, inasmuch as they say that these properties "are not sufficient to explain its other real or fancied curative properties."

Although cod-liver oil has been used very largely in the treatment of rickets, there has not been complete unanimity of opinion regarding its curative properties. Some have regarded it as most effective. Perhaps it is fair to say that the majority have looked upon cod-liver oil as a substance which was of some value in rickets but in no way to be considered as a specific. To this latter class we confess that we ourselves have belonged.

Metabolism experiments, especially those of Schabad, have seemed to show definitely that cod-liver oil is of value. Schabad [3] found that cod-liver oil alone increased the retention of calcium in rickets, that sesame oil had no effect, that phosphorus alone had no effect, that cod-liver oil and phosphorus had a greater effect than cod-liver oil alone. These results have been essentially confirmed by Ernst Schloss.[4]

There are certain objections that can be raised against conclusions drawn from metabolic studies in almost any chronic disease. The results are determined during only a very small fraction of the total disease period and may not indicate satisfactorily what is going on during all of the time. Moreover, with calcium determinations it is a well-known fact that, owing to the large amount of calcium in the stools, erroneous conclusions may be reached, unless the stools for the period of analysis are sharply marked off; but the accomplishment of this is not always an easy matter. It seemed to us that direct ocular proof regarding the effect of cod-liver oil could be furnished by means of radiograms. We have not been able to find that the influence of cod-liver oil upon rickets has previously been determined in this way. Phemister [5] had used radiograms to follow the effect of the ingestion of phosphorus on different diseases involving bones. Since the appearance of the preliminary report of our work [6] he has published a paper upon the influence of phosphorus in rickets.[7] In a few instances the phosphorus was combined with cod-liver oil. He did not use cod-liver oil alone.

The experiments were planned to be as simple as possible. All the patients were in the hospital during the period of

observation, because our experience had taught us that the administration of cod-liver oil was too uncertain if left to parents at home. In so far as possible, and it was usually the case, the children were maintained upon the same diet as that upon which the rickets had developed. This was chiefly milk and cereal. The diagnosis of rickets was made by physical examination and by means of radiograms. As soon as the latter had been obtained, plain cod-liver oil was given in amounts varying from 2 to 4 c. c. three times a day. From time to time radiograms were taken. The rickets in all our cases was severe. In some it had existed for a long time without treatment.

What are the criteria upon which a diagnosis of rickets can be made from radiograms? They are to be found (a) in the shaft and (b) at the extremities of the long bones. With rickets there is a reduction, more or less, in the inorganic material of the bone. This is the substance which is impervious to the X-ray, and to the extent that this material is reduced, therefore, the shaft becomes less capable of casting a shadow. One of the most distinctive appearances of rickets is a reduction in the density of the shaft. In mild rickets the change is difficult to detect, in the more severe it is unmistakable. The cortex does not appear homogeneous and solid, but porous. As the trabeculæ which compose it have a parallel longitudinal arrangement, the cortex may appear lamellated. By the reduction of inorganic material the bones are weakened. Curvatures of the long bones are frequent and fractures much more common than would be supposed from clinical examination. The fractures are frequently subperiosteal without separation of the fragments. Callus formation is strikingly small in amount.

The configuration of the shaft of the bone differs very greatly according to the duration of the disease. In young, especially premature, infants the shafts are very thin and delicate and the cortex of linear thickness. In children of the second and third years the shafts are frequently increased in diameter and may have lost much of the normal form. The cortex is often of great thickness. This latter change is probably due to the fact that, in a conservative manner, nature attempts to compensate for the weakness of the shaft by a thickening of the periosteum. With the attempts at repair and the relapses that undoubtedly take place through months and perhaps years (for rickets is by no means necessarily a continuous process), more and more thickening of the periosteum results and more and more of the new material is by metaplasia transformed into bone.

In the normal bone the transition from the shaft to the cartilage is abrupt. Under all circumstances the end of the normal bone is sharply outlined. The earliest rachitic lesions at the junction of cartilage and shaft cannot be detected by means of the X-ray. As the process advances, a change is noticed at the cartilage-shaft junction as an indistinctness in the outline of the end of the shaft, which becomes progressively less clear cut. The shaft seems to terminate in thin, thread-like prolongations, which together give an appearance somewhat resembling a fringe. In the most advanced cases the rachitic "fringe" may be several millimeters long. In many children suffering from rickets cupping of the ends of certain of the long bones can be demonstrated. Just before its termination the shaft becomes broadened and at its line of junction with the cartilage forms a crater, often of considerable depth. When the end of the shaft has this concave appearance, it is usually possible to trace out the circumference of the crater. The cupping is much commoner at the ends of some bones than others. The lower end of radius and ulna and the upper end of the fibula are especially apt to be affected. In some of the most pronounced cases of rickets not only is no cupping present, but there is no enlargement at all toward the extremity of the bone. In these cases the shaft seems to terminate in an irregularly shaped stump. The reason for the peculiar appearance is that the new bone formed after the initiation of the rachitic process, free from lime salt deposit and therefore not capable of casting a shadow which distinguishes it from the surrounding soft parts, is very extensive. The shaft does not end where it ceases to be visible with the X-ray. It is prolonged onwards in the direction of the epiphysis, but is of such a composition as to cast no distinguishable shadow. When the rachitic process is as extensive as here described, there seems to be an abnormal distance separating the ends of the shafts at the joints, as, for example, the femur and tibia at the knee. If the rachitic process at the junction of cartilage and shaft is extensive, the center of ossification may not be visible at all, or it may appear much smaller than it should be to accord with the age of the subject, or its outline may be extremely faint. The epiphysis may be dislocated or at least bent over to one side. The changes at the junction of cartilage and shaft are much more marked at the ends of some of the long bones of the extremities than of others, but in advanced rickets changes at the junction of cartilage and shaft can be seen in all the long bones.

After treatment with cod-liver oil changes are detected by radiogram about the end of the third or fourth week, occasionally a little before. It is true that the deposition of salts must reach a certain magnitude before the deposits are detectible by so coarse a method as the X-ray. We have, nevertheless, some information in addition to that afforded by the X-ray to show that salts are not deposited immediately. One child died of an intercurrent disease six days after and another twelve days after the beginning of cod-liver oil medication. In the bones of neither of these children was there any deposition of calcium salts appreciable on microscopical examination. It is probable that in some children, particularly those in whom the disease is just entering upon a stage of repair, the deposition of lime salts occurs more readily than in those who are in the midst of an active and advancing rickets. The first evidence of repair is usually in the form of a line, always broken, beyond the extremity of the shaft, between this and the center of ossification, which latter may or may not be visible. A relatively clear space is often left between this line and the end of the bone. As time advances, this new line becomes thicker and thicker, and more dense than other parts of the

Fig. 1.

Fig. 2.

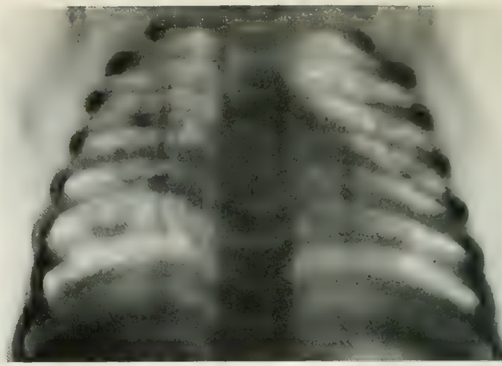
Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.



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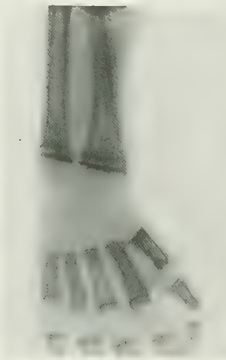

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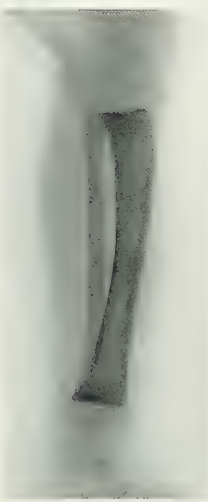

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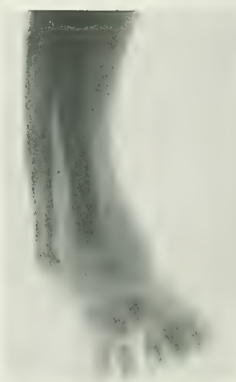

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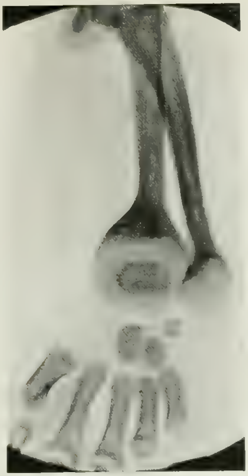

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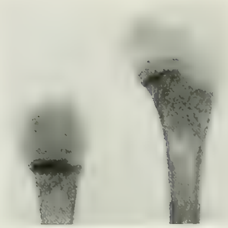

Fig. 16.

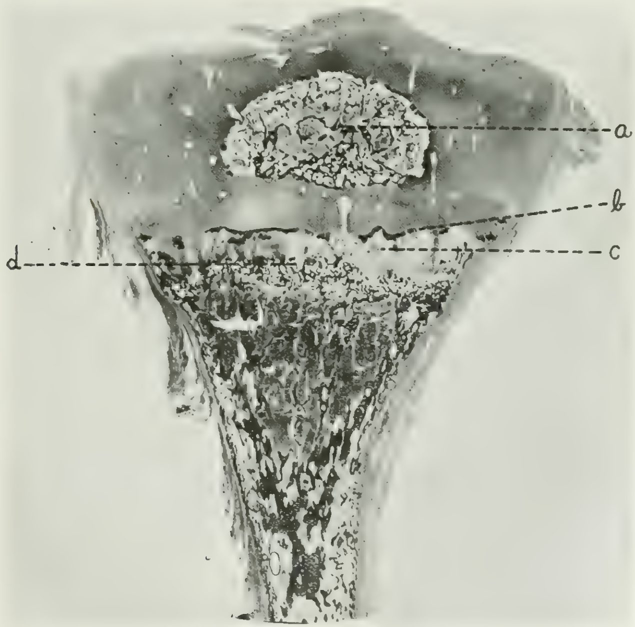

Fig. 17.

Fig. 18.

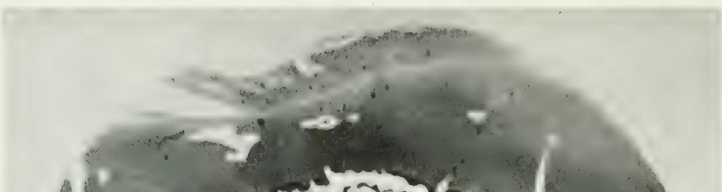

Fig. 19.

bone. Gradually the clear area between this line and the end of the shaft becomes filled in, until it can no longer be appreciated. At times there is not this linear formation, but the whole extremity of the bone which has before been invisible gradually comes into view. Pathological studies, chiefly by Schmorl,[8] have shown that in the process of healing the deposition of calcium salts takes place first in the cartilage on the epiphyseal side of the transitional zone, that is, in the place where the lime salts would have been deposited provided there had been no rickets. The deposition in this situation is the cause for the linear shadow in the radiogram. Gradually, the material in the transitional zone is transformed into bone and becomes infiltrated with lime. The repair of the transitional zone is time-consuming. When the rachitic process has been of such duration and is so extensive that the shaft seems to terminate in a stump, under cod-liver oil administration the true end of the bone gradually becomes visible. The deposition of lime salts frequently takes place in such a manner as greatly to increase the crater-like appearance. The end of the shaft seems like a cup or socket embracing the center of ossification. At the end of two or three months calcification of the ends of the bones seems to be complete. The finer architecture of that portion of the shaft which was the seat of the rachitic process is not, however, normal. The normal arrangement of the trabeculæ has not been restored.

As the result of the deposition of lime salts in the transitional zone the long bones seem to have grown greatly in length in a very short time. They probably *have* grown somewhat under the influence of the cod-liver oil. Very little of the apparent increase in length is, however, real. The portions that have been unable to cast shadows before are now plainly visible in the radiogram. The potential has been transformed into actual lime-containing bone.

Swelling of the costochondral junctions which may not have been demonstrable by radiogram before the administration of cod-liver oil can readily be detected in many instances. The swellings appear like buttons on the surface of the chest. The administration of cod-liver oil does not cause the enlargement of the costochondral junctions to diminish rapidly in size. On the contrary, when infiltrated with lime salts in this way, they must become more and more firm for a considerable period of time.

Increase in the density of the shaft takes place coincidently with the changes at the extremities, but it is a more slowly developing process, so that the bone may still be distinctly less dense than normal at the end of three months, when the extremities are, so far as can be determined, entirely calcified. Periosteal thickening, whether previously demonstrable or not, becomes strikingly apparent as the result of cod-liver oil medication. In extreme cases even the trabecular arrangement of the thickened periosteum can be appreciated. In contrast to the longitudinal arrangement of the trabeculæ of the cortex the periosteal trabeculæ are frequently arranged so that they are perpendicular to the long axis of the bone. When there have been fractures, the evidence of callus formation is usually greatly increased. The centers of ossification, previously invisible or faint, become clearly outlined. They also increase in size.

In our studies which have comprised in all some 50 cases, the results have been uniformly consistent. We feel justified in saying very definitely that cod-liver oil brings about a change in the bones which, if the diet be not too faulty, amounts to complete cure. The change is not noticeable at once, but is readily demonstrable in almost all cases by the end of a month. In two or three months so much infiltration with salts has taken place that the extremities of the bones, except for deformities, are practically normal, and only differences in the finer architecture of the ends of the bones indicate the previous existence of a rachitic process. We look upon cod-liver oil as a specific for rickets. We have not seen it fail in any single instance and we have known it to cure the rickets even though the children were dying of some other disease. Thus, one child with a sarcoma lost the radiographic evidences of rickets, though succumbing to the malignant growth, and another child, who was hanging between life and death as the result of a severe thoracic involvement and who finally died of pneumonia one month after treatment with cod-liver oil, did not fail to show calcium deposition in the bones both by radiograms and by microscopical examination. We know of hardly another drug that in disease exerts so regular, certain and specific an effect as does cod-liver oil in rickets.

Our thanks are especially due to Miss Mary S. Smith of the X-ray Department of the Johns Hopkins Hospital for her constant interest and cooperation in procuring the radiograms.

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DESCRIPTION OF PLATES

Fig. 1.—A. T., aged 13 mos., colored. Before treatment.

Fig. 2.—A. T. After treatment with cod-liver oil for 1 mo. 24 days. The actual terminations of lower ends of radius, ulna and humerus, previously invisible, have become visible. Apparent dislocation of epiphysis of radius. Marked periosteal thickening. Centers for carpal bones larger and more distinct.

Fig. 3.—A. T. Before treatment. Shafts of tibia and femur terminate abnormally. No enlargement towards epiphysis. Appearance as if ends of shafts had been sheared off. Femur is fractured. Incomplete fracture of tibia. Cortex of tibia has characteristic porous appearance. Periosteum does not appear thickened.

Fig. 4.—A. T. After treatment for 1 mo., 24 days with cod-liver oil. The fan-shaped lower ends of femur and tibia have come into

view. The old blunt terminations of the shafts are still visible. Great thickening of the periosteum of tibia, fibula and femur. Nuclei of ossification greatly increased in size. Apparent growth of bones in length.

Fig. 5.—H. G., aged 10 mos., colored. Before treatment. Great rarefaction of all bones. Nucleus of lower epiphysis of humerus and radius invisible. Slight evidence of periosteal thickening.

Fig. 6.—H. G. After treatment with cod-liver oil for 42 days. Apparent increase in length of radius and ulna. Deposit of salts in previously uncalcified portions of shaft renders these portions capable of recognition. Dark, irregular line at lower ends of shafts of radius and ulna caused by dense deposit of salts in portion of cartilage bordering on transitional zone. Transitional zone itself still incompletely calcified. Architecture of this zone different from that of shaft. Centers of ossification of lower epiphyses of radius and humerus visible. Extreme degree of periosteal thickening now evident. Bones much more dense.

Fig. 7.—H. G. Before treatment with cod-liver oil. Costochondral junctions cannot be made out.

Fig. 8.—H. G. After treatment with cod-liver oil for 42 days. Costochondral junctions now plainly visible. On the right side they appear as rings.

Fig. 9.—H. M., aged 11 mos., colored. Before treatment with cod-liver oil. Carpal bones invisible. Note distance from ends of radius and ulna to metacarpal bones.

Fig. 10.—H. M., after treatment with cod-liver-oil for 28 days. Dark, irregular, horizontal lines at extreme ends of radius and ulna mark calcium deposits in zone of cartilage on distal side of transitional zone. Transitional zone, still uncalcified, pale in contrast. Periosteal thickening striking. Two centers of ossification for bones of carpus visible. With apparent lengthening of radius and ulna, distance between them and metacarpal bones is noticeably diminished. Metacarpal bones share in the changes.

Fig. 11.—H. M., after treatment with cod-liver oil for 1 mo. 24 days. Density of all bones greatly increased. Calcification of lower ends of radius and ulna probably complete but disarrangement of finer structure of bone still remains.

Fig. 12.—J. J., aged 10 mos., colored. Before treatment.

Fig. 13.—J. J., after treatment with cod-liver oil for 1 mo. A faint, broken line caused by a deposit of lime salts marks the junction of cartilage and transitional zone. True length of shafts of bones now appreciable. Changes extremely early.

Fig. 14.—M. C., aged 21 mos., colored. Before treatment with cod-liver oil. True ends of shafts of radius and ulna not visible. Both bones look as if cut off.

Fig. 15.—M. C. After treatment with cod-liver oil at home intermittently for six or more months. (Case does not belong to hospital series.) Both radius and ulna now terminate in huge bulbous enlargements. That of radius seems almost to envelop large nucleus of ossification.

Fig. 16.—J. J. Upper ends of femur and humerus freed from soft tissue. Deposits of lime salts in cartilage on epiphyseal side of rachitic transitional zone are clearly visible. Deposits are fragmentary and hence the line is irregular. Characteristic porosity of cortex.

Fig. 17.—J. J. Section through upper end of tibia 200 microns thick. Stained with H. and E. and silver nitrate. Section cut slightly on the bias.

(a) Nucleus of ossification incompletely rimmed round with freshly deposited line of calcium phosphate.

(b) Broken line of calcium phosphate deposition at junction of cartilage and transitional zone.

(c) Transitional zone composed of cartilage, osteoid, connective tissue, blood vessels and marrow. No one of these elements casts a shadow distinguishable from those cast by the soft tissues. This zone, except at periphery, still free from calcium.

(d) Termination of that portion of shaft which was visible in radiogram.

Fig. 18.—J. J. Higher power magnification of calcium deposit in proliferative cartilage bordering on transitional zone.

Fig. 19.—J. J. Higher power magnification of nucleus of ossification. Enlargement of nucleus of ossification, seen in radiograph after treatment with cod-liver oil, due to formation of a fresh line of calcium phosphate deposit outside old.

LIPODYSTROPHIA PROGRESSIVA*

WITH REPORT OF A CASE

By Henry Lee Smith, Baltimore

Lipodystrophia progressiva is the name given by Arthur Simons[1] of Berlin to an uncommon disorder, a case of which he reported in detail in 1911.

The affection is characterized by a symmetrical, slowly progressive and almost complete disappearance of the fat from the subcutaneous tissue of the head, face, neck and upper extremities, and also from the trunk as far as the pelvic bones and folds of the groin where the fat absorption abruptly ends. This peculiar form of wasting is first noticed in the face whence it creeps downward over the parts of the body mentioned.

All cases of lipodystrophia, however, do not conform to the description just given, for in a few instances—in the male almost exclusively—the emaciation remains limited to the face and neck, or to the face, neck and thorax, as is shown in the cases reported by Husler,[2] Hertz and Johnson,[3] Batty Shaw,[4] Weber[5] and others. Likewise in females, there may be variations as to the parts of the body showing fat atrophy. In Lewandowsky's[6] case, for example, the face and trunk were atrophied but not the arms. In cases reported by Barraquer,[7] Boissonnas[8] and Herrman[9] there was no loss of fat in the abdominal walls, and in one case, that reported by Laignel-Levastine and Viard,[10] the face, apparently, was unaffected.

The term lipodystrophia progressiva is not altogether an appropriate one, for the buttocks and lower extremities do not waste, but on the contrary show an excess in the accumulation of subcutaneous fat, such as is seen in many women at middle-age. This fat increase in the lower parts of the body, however, occurs almost wholly in the female. It is symmetrical in distribution, invading first the buttocks and then, successively, the thighs and legs. Exceptionally, there may be a decided asymmetry in the contour of corresponding parts, as is well shown in the illustrations of Laignel-Levastine and Viard's[10] case. The feet and ankles are not implicated.

* This article was read in abstract, and the patient exhibited before the Baltimore Medical Society, Nov. 5, 1920.

In the 26 characteristic cases of progressive lipodystrophy found in the literature, *i. e.,* the cases showing both the atrophic and hypertrophic features of the disease, only two of the patients were males, a boy six years of age, whose case was reported by Boissonnas,[6] and Gerstmann's [11] patient, a soldier 32 years of age. Both of these reports were published in 1916, and until that time most of the authors had seemed inclined to regard the predominance of the gluteofemoral fat as belonging to the female sex. Spear [12] ventures the opinion, that "this characteristic is accentuated by the efforts made to over-nourish the patient to counteract the wasting which takes place in the upper portion of the trunk, face, etc." It is well known that excessive fat of the buttocks, "steatopygia," is seen as a normal development in the female of certain African races.

The increase in the size of the buttocks is usually not noticed until some years after the onset of the facial wasting. Feer [13] thinks it is possible that the fat atrophy and the fat hypertrophy begin simultaneously and progress concurrently, and that early evidence of gradual enlargement of the lower regions of the body might be obtained by accurate measurements. In Simons' [1] first case, the obesity of the lower parts of the body apparently had preceded by several years any wasting of the upper parts. In one case, that of Laignel-Levastine and Viard,[10] the legs first became enlarged, then the thighs and, finally, the buttocks.

The first case of the disease was published by Barraquer [7] of Barcelona in 1906 under the heading, "Histoire clinique d'un cas d'atrophie du tissu cellulo-adipeux," but as far back as 1895 Sir William Osler, when in America, had observed the characteristic features of this singular malady in a girl of 10 years of age. The wasting had begun in the face at the age of five years and had been followed by extreme emaciation of the trunk and upper extremities. It was ascertained in 1913, that the emaciation had remained practically at a standstill, but that the patient had enjoyed good health, and at the time of inquiry, felt well and was actually stronger than most women. Sir William Osler did not himself report the case, but gave Dr. F. Parkes Weber [5] permission to record it.

In 1907 Harry Campbell [14] exhibited a typical example of the disorder before the Clinical Society of London, which he described as a "Disappearance, more or less complete, of the subcutaneous fat above the region of the lower extremities." Pic and Gardère [15] reported, in 1909, "Un cas d'atrophie généralisée de la face et de la région sus-ombilicale du corps avec pseudohypertrophie de la région pelvienne et des membres inférieurs."

Simons [1] applied the term "lipodystrophia progressiva" to the case reported by him in 1911, and since then the disease has become known by that name. Gerhartz [16] thinks the epithet misleading. He preferred to call the case, published by him in 1916, "lipodystrophia progressiva superior." E. Feer [13] of Zurich, in an elaborate article which appeared in 1915, reported two cases, one of which had been referred to him by Boissonnas. The last publication that I have seen on the subject is a brief description of a case in a boy reported by Leipoldt,[18] of South Africa, in February, 1921.

The literature was reviewed by Weber [5] in 1917 and by Boissonnas [8] in 1919. Three Americans, Herrman,[9] Spear [12] and Epler [17] have reported one case each. About 27 well-defined cases have been published, and, additionally, about half as many of the limited or incomplete form. Of the former group, which is made up of 25 females and 2 males, the cases are presented more or less in detail and the diagnosis seems clear, but in the latter group, all of whom are males, the diagnosis of some of the cases is made on insufficient findings, and is therefore, to say the least, of questionable value.

The general appearance of a well-marked case of lipodystrophia progressiva in the female is grotesque. The eyes are usually set back. The malar arches stand out between the sunken temples and hollow cheeks. The face is deeply furrowed when the patient smiles. The neck is thin and stringy looking. The clavicles, scapulæ, and vertebral spines become prominent, and the intercostal spaces are plainly visible. The muscles beneath the lean skin are clearly outlined when made tense, giving the impression of over-development. The breasts are hard and pendulous. The thin abdomen and the conspicuously obese buttocks, thighs and legs complete the incongruous picture.

The onset is insidious. There is no fever or other constitutional disturbance of consequence. In the early stages of the atrophy, there may be vague aches and some malaise, and later a sensation of chilliness, hyperidrosis and slight nervousness.

Because of the emaciated condition, tuberculosis is generally suspected and the patients themselves naturally become apprehensive as to their condition and sensitive as to their appearance, but a definite causative relationship between progressive fat dystrophy and mental change is lacking. The functions of the cranial and peripheral nerves may be regarded as normal. Such clinical phenomena as exaggerated tendon reflexes, abolished unilateral plantar response, facial tic, diminished pupillary response, unilateral tremor and nystagmus, which have been recorded in isolated cases, are more probably due to factors other than those concerned with the causation of lipodystrophia. All investigators admit the absolute soundness of the muscular and osseous systems, and also, the normal muscle and nerve response to electric stimulation.

The digestive, respiratory, renal and cardiovascular systems are not abnormal. The blood picture proves negative.

The skin of the lean areas is natural in color, smooth, soft and freely movable over its subadjacent plane. It forms a thin fold when picked up, and when stretched, shows no loss of elasticity.* There is usually no disturbance of the cutaneous

* Had the cases so hastily reported as probable lipodystrophia in the male been more carefully examined, doubtless some of them might have been proven to be identical with that even rarer condition known as "elastic skin," examples of which are seen in the so-called "elastic-skin men" or "India-rubber men" of the circus side-shows. The cause of the extreme extensibility of "elastic skin" is not clear, and more or less confusion exists as to its histopathology, which only quite recently has been studied by Duhring, Kopp, Seifert, Du Mesnil and

secretions. Campbell[14] reported a foul smelling sebum in his case, and Feer[13] refers to a "peculiar smell" which persisted in his patient even after repeated bathing.

Examinations of pieces of skin and subcutaneous tissue removed from the emaciated areas in the living patient are recorded by Simons,[1] Christiansen,[19] Feer[13] and for my own case. The findings are uniformly the same. The epidermis and corium are normal, and the one histologic change is practically the entire absence of fat in the subcutaneous tissue. Simons,[1] in 1913, in a thorough study of his first patient, clearly demonstrated this lack of fat by an excellent illustration of a section of skin and subcutaneous tissue removed from the thorax, near the axilla, and, by way of contrast, he showed in another illustration the abundant presence of subcutaneous fat in a section from a piece of the scalp taken from a healthy, but not obese man, 34 years of age. He pointed out further that a piece of skin excised from the buttocks of his patient was macroscopically and microscopically normal. It is known that one to several millimeters of fat remain beneath the skin of greatly emaciated individuals suffering with such disease as tuberculosis, cancer and the like.

The various metabolism tests including the metabolic rate were found to be practically normal in Simons' case, but in my case the CO_2 output exceeded the normal by 36.8 per cent. In testing out the fat metabolism, Feer found no lipemia after his patient had been given a meal of 200 gm. of 20 per cent cream, and in Simons' first case there was no increase in the lipase of the blood.

Further investigations by Feer showed that a subcutaneous injection of pilocarpin produced only slight moistness of the cheeks, but that there was a copious outbreak of sweat on the chest, back, arms, forehead, belly and lower extremities. The pulse, respiration and intestinal peristalsis were uninfluenced. A subcutaneous injection of one milligram of adrenalin produced after three minutes a rise of 50 points in the blood pressure. No glycosuria followed the ingestion of 100 gm. of glucose.

There is no general enlargement of the lymph nodes. Moderate increase in size of the thyroid with evidence of more or less over-activity of this gland has been noted in a number of cases.

Two autopsies have been reported. The first was in the case of Husler's[2,5,6] patient, an undersized boy, who died in his 14th year from epidemic cerebro-spinal meningitis. At six years of age he showed signs of bifacial atrophy. The wasting, however, remained limited to the face. The muscular, osseous and nervous systems were normal. The autopsy was done mainly with reference to the meningeal condition. As regards the endocrine glands, the thymus was present, the genitalia were infantile, the suprarenals were found enlarged and contained a whitish medullary substance and a cortex rich in fat and pigment.

The second autopsy was reported by Weber and Gunewardene[20] in the case of a girl previously reported by them as showing a well-marked lipodystrophia progressiva. Death occurred at the age of 13 years from pyemia following an operation for mastoid disease. At autopsy the body was greatly wasted from the prolonged illness. Fat was found to be practically absent from the subcutaneous tissue of the parts of the body above the pelvis, but was present in moderate amount in the gluteal regions, orbits, omentum, about the kidneys, heart, pericardium and under the serous membranes. The authors felt, that had the child died from some disease not associated with extreme emaciation there would have been much more fat remaining in the regions not affected by the lipodystrophia. Microscopical sections of one ovary, the pituitary gland and both suprarenal glands showed nothing remarkable, and there was no microscopic subcutaneous fat found in a piece of the anterior abdominal wall, nor in a piece of the hairy scalp taken from the occipital region. The thyroid was relatively rather large and gave macroscopic and microscopic evidence of over-secretion of colloid material; but this did not exceed in amount that physiologically present at the age of puberty. The thymus had almost entirely disappeared. The brain was normal. There was no evidence of tuberculosis.

Etiology.—The cause of lipodystrophia is unknown. Tuberculosis, syphilis and alcoholism may be disregarded as factors. The disease is not hereditary. There is no racial predilection. The age of onset is usually about the sixth year, or shortly before puberty, and, as already shown, females are more frequently and characteristically affected than males.

Spear[12] thinks the condition closely related to the muscular dystrophies. Most authorities, however, concur with Simons[1] and Feer[13] in the opinion that it is due to a disturbance of secretion of the endocrine glands. Boissonnas[8] believes an obscure lesion of the central nervous system to be the cause. He finds it difficult to explain how a glandular lesion can, at the same time, account for the absence of fat in one part of the body and an excess of it in another; and adduces in support of his view the admission of both Simons and Feer that, whatever the cause, the nervous system must be, at least, a contributing factor. Herrman[9] thinks it quite possible that some substance circulating in the blood could produce just such opposite conditions as are met with in progressive fat dystrophy. He refers to the selective action that some organisms, toxins, and other substances have for certain tissues of the body.

Feer emphasizes the possibility of thyroid dysfunction as causative. He calls attention to the thyroid influence on the panniculus adiposus in thyrogenous obesity, on the one hand, and to the fat deficit in Basedow's disease on the other. He further alludes to the myxedematous state of the subcutaneous

others. In this condition the elastic fibers are described as elongated, but unchanged in number and diameter. The derma itself is found to be undeveloped or myxomatous; the fibrous connecting tissue is described by some investigators as absent, by others, as greatly modified; the nerves and blood-vessels are elongated and winding, and, according to Williams and Unna, there is an increase in the muscle fiber. Unna has found the marked splitting of the collagenous substances which he regards as an important factor in producing the usual extensibility of the skin. (Stelwagon, Diseases of the Skin, W. B. Saunders Co., 1916, 636; *also*, Diseases of the Skin, Ezekiel Hartzell, J. B. Lippincott Co., 1917, 518.)

connective tissue in athyrosis. The decided increase in the subcutaneous fat in certain parts of the body following castration in both sexes, and the general adiposity associated with hypoplasia of the genitalia as seen in the Froelich syndrome, are further examples of endocrinopathic influence on the panniculus adiposus.

Other authors, besides those last mentioned, have noted in their cases certain symptoms which are usually interpreted as suggestive of over-function of the thyroid gland. It is well therefore to study carefully all cases of lipodystrophia with reference to this gland as a possible factor of the disease. In my own case there are present, moderate enlargement of the thyroid, some prominence of the eyes, slight lagging of the upper lid, moderate tachycardia, tremor of the fingers, hypertension, acrocyanosis, slight nervousness and a decided acceleration in the metabolic rate. Another case of lipodystrophia now under observation by Dr. L. F. Barker and Dr. T. P. Sprunt, shows features which might be interpreted as due to hyperthyroidism, namely, marked acceleration in the basal metabolism, hypersensitiveness to adrenalin, moderate eye signs of hyperthyroidism, fine tremor of the fingers, marked under-nourishment, but no tachycardia and no thyroid struma.

It is quite apparent that any causative relationship existing between perverted action of one or more of the endocrine glands and certain metabolic disorders cannot be postulated until we know just what are the individual and interdependent functions of these glands in maintaining the normal metabolic balance, and that without such knowledge the cause of lipodystrophia must remain obscure.

Diagnosis.—Lipodystrophia is probably not so rare a condition as it was at first thought to be. The diagnosis is based on the symmetrical wasting of the face, or face and upper parts of the body associated in females, and exceptionally in males, with an increase in the size of the lower parts. The good health of the patient, the biopsy findings, the negative radiographs, the retention of the normal muscle and nerve functions, the negative electrical reactions and the normal sexual development exclude the other trophic disturbances.

Tuberculosis, cancer and other wasting diseases are readily eliminated by the excellent general condition of the patient. The marked emaciated appearance of the face in an individual enjoying good health is practically pathognomonic.

It is a point of some diagnostic importance to note that the buccal fat, or sucking pad, which is lost in lipodystrophia, is retained in infantile atrophy. The preservation of this subcutaneous cheek fat accounts for the rotundity of the face not infrequently seen in children otherwise greatly emaciated in advanced pulmonary tuberculosis.

The outlook in regard to the life of the patient is quite favorable. Death has been reported in two cases which was due to intercurrent disease. The atrophy in Campbell's [14] case progressed at the rate of about 2.5 cm. yearly. After a period, varying from 10 to 20 years, the maximum of the fat atrophy is reached. The spontaneous reappearance of fat in the lean parts has not been recorded. The increase in the lower part of the body also seems to remain at a standstill after progressing for a certain number of years.

The *treatment* has been unsuccessful. It has included the administration of thyroid, pituitary and ovarian extracts, massage, electricity, hydrotherapy and overfeeding. In the case of Pic and Gardère [15] rest and feeding resulted in some increase of the fat in the lower extremities, but the atrophied areas remained unchanged. Holländer [21] obtained temporary cosmetic improvement in Simons' [1] first case by the subcutaneous injection in the face of a mixture of human and sheep fat, and transient improvement was also obtained in Christiansen's [10] case by the injection of paraffin.

REPORT OF A CASE

The patient, Mrs. C. R., aged 30 years (F. 67788), is an intelligent woman of German descent. She was first seen by me on February 12, 1920, in the Out-Patient Department of The Johns Hopkins Hospital. She complained of sunken cheeks and thinness of her neck, body and arms. The object of her visit was to see if something could be done to "fill out" her cheeks. She had been told by her private physician that she had "muscular atrophy."

Family History.—Her father died of tuberculosis of the lungs. Her mother died of cancer of the stomach. The patient is the youngest child of a family of ten. Her birth was normal. Three brothers and three sisters died during infancy from diseases unknown to patient. Two sisters are living and well. One brother, previously tuberculous, is now in good health. None is excessively thin or fat and the patient says that there has been no affection in the family similar to her own.

Personal History.—The patient had measles and mumps in childhood. Menstruation began at the age of 15 years and has been normal. She is the mother of a healthy boy and has not miscarried. She has drunk beer in moderation. She tells me that she was treated in The Johns Hopkins Hospital Dispensary in 1904, and that because of her emaciated appearance, it was thought at that time that she probably had consumption of the lungs. In 1917 she again reported at the Dispensary complaining of a "cold." The diagnosis then was, "Chronic nephritis and pulmonary tuberculosis (largely fibroid)." She has never had a continuous cough, chest pain, or spitting of blood. She has frequently been regarded as cancerous or consumptive by her neighbors and friends. Her health, however, has been exceptionally good, and she has never failed to do her household work.

The wasting began in her face when she was a rosy, round-cheeked girl, about 14 years of age. It was accompanied by a vague aching in her face, sleepiness and a feeling of general weakness. These symptoms soon passed off, but the wasting progressed slowly downward, ending abruptly at the pelvic bones and groin folds. Her face, neck, thorax, upper extremities and abdomen became involved in the order named. Within three months' time from the onset, her face (she says) reached the present degree of emaciation. She does not know how long it took to grow thin from her face to her groins, but she recalls that she became fatter in the buttocks and lower extremities shortly after her face began to waste. She feels perfectly well and regards herself as stronger than most women, although she tires rather easily and is somewhat nervous at times. Her appetite and digestion are good and her bowels are regular. She voids once during the night after retiring. In spite of her facial disfigurement, she is cheerful and happy in her home.

Physical Examination.—Height, four feet, eleven and a half inches (151 cm.), without shoes: weight, 101 pounds (46 kg.), with clothes.

Appearance.—The striking feature at a glance is the emaciated appearance of the temples, face, neck, thorax, abdomen and upper extremities, contrasting sharply with the plumpness of the lower part of the body which begins abruptly at the groin folds and pelvic bones and includes the buttocks, thighs and calves. The

ankles and the feet are not abnormal. The facial wasting gives the patient an old, uncanny, even cadaverous look. Smiling produces deep folds under the zygomas and around the angles of the mouth. There is slight prominence of the eyes and of the thyroid gland, and marked prominence of the muscles of the face, neck, trunk and upper extremities.

Skin.—The skin is soft, not inelastic and of normal color except for moderate pallor of the face. There is no dermatographia. The hair growth is normal. Over the affected parts the skin can be easily picked up in thin folds and stretched away from the underlying muscles. Over the buttocks, thighs and calves it is firm and dimples on pinching. The skin-fold of the cheeks measures 3.5 mm., and of the left buttock 45 mm.

*Measurement of Skin-folds.**—Cheeks 3.5 mm., breasts 3 mm., neck 3 mm., mid right arm 3 mm., mid left arm 2.5 mm., mid right forearm 3 mm., mid left forearm 2 mm., upper back 4 mm., lower back 5 mm., abdomen 4 mm., right buttock 40 mm., left buttock 45 mm., outer, mid right thigh 25 mm., left thigh 31 mm., right calf 19 mm., left calf 35 mm.

Microscopical Examination of Skin.—A piece of skin with underlying tissue down to and including the fascia was received in formalin. It was cut from the left upper forearm. A portion was immediately transferred to Zenker formol and another to Marchi's fluid. Later, frozen sections were made from the formalin tissue and stained with sudan, with hæmatoxylin and eosin, with Wright's stain and with Weigert's elastica stain. Sections from Marchi's fluid were stained with safranine and also with the elastic stain and Mallory's connective-tissue stain. These were repeated with the Zenker section which also gives an especially brilliant Wright's or Giemsa's stain.

The skin itself was normal in appearance in gross except that the subcutaneous tissue was loose and soft in texture, grey and devoid of fat. The fascia, which was only a tiny fragment, seemed normal. Sections stained by the above methods show the following: The epidermis appears to be normal. The epithelial cells are in a uniform layer with normal papillæ of the corium and the normal arrangement of hair follicles. In one section there was a minute area in which the epithelium was somewhat pigmented, and in the interpapillary areas there were groups of pigmented cells. Elsewhere the corium shows no accumulations of cells except in one or two papillary projections which are infiltrated with mononuclear cells. Most of these are small lymphoid cells, with no granules, but there are a few scattered eosinophile mononuclear cells. Everywhere through the corium in much greater numbers than in the normal specimens used for comparison, there are twisting and writhing elongated cells which appear to be wandering in the crevices of the connective tissue, especially about the blood vessels and sweat glands, but also about the sebaceous glands. These have a relatively small nucleus and an elongated cell body full of basophile granules (mast cells). The corium itself is not abnormal; the elastic tissue fibrils run in alternate directions in successive layers and are very well developed. The hair follicles are normal in all respects and the sebaceous glands are quite normal. They contain much doubly refractive fat in large and small globules, which shines with a marble-like luster under the polarizing microscope. It stains black with osmic acid and red with sudan. Some of it is stained adhering to the hairs as they lie in the root sheaths. The sweat glands are abundant and normal. The tissue just below the corium is extremely loose and is composed of fine, wavy, connective tissue fibrils by no means so coarse as those of the corium, and a few delicate elastic fibrils. There are, as in the corium, blood vessels and some small nerves which appear to be normal. The deeper layers of this tissue contain more vessels of larger size, of which the arteries show a distinct elastic lamella. The veins show no obvious elastica but their endothelium appears to be rather swollen and extremely transparent. No fat is found in the upper layers of this tissue and it is only in its deepest layers that one encounters several small groups of three or four fat cells which are distended with fat globules. This stains brilliantly with osmic acid or sudan. It is not possible to distinguish which of the other cells about these and supplied by the same small blood vessels are really fat cells, since none of them have any very characteristic form and none contain oil droplets. The fascia does not seem to be abnormal.

Summary.—The skin is normal in histological structure except that there is an almost complete absence of fat in the tissue beneath the corium. Fat persists in the sebaceous glands where part of it is doubly refractive. There are very numerous mast cells in the corium and even in lower layers of the tissue. (W. G. MacCallum.)

Muscles.—The muscles are everywhere normally developed and are strong to test. In the lean areas they are plainly outlined when put in action. There is no fibrillary twitching. The reaction to electrical stimulation is normal.

Extremities.—Because of the absence of the subcutaneous fat, the upper extremities, though well muscled, appear markedly wasted when compared to the plump lower extremities. The hands seem atrophied. They are moist, clammy and somewhat cyanotic. There is slight tremor of the fingers. The buttocks, outer thighs and calves are well padded with subcutaneous fat. The knees are dimpled, and the ankles and feet are normal. In a word, the panniculus adiposus seems to have slipped from the upper part of the body to the lower extremities, there being a line of sharp demarcation between the areas of fat atrophy and fat hypertrophy at the upper margin of the pelvic bones and at the groin folds. The circumference of the left calf is greater than that of the right.

Measurements.—Head, 49.5 cm.; neck 28.5 cm.; chest (over mammary glands) 77 cm.; biceps, right, 21.5 cm.; left, 20 cm.; forearm, right, 21 cm.; left, 21 cm.; wrist, right, 13.5 cm.; left, 13.75 cm.; waist, 58.5 cm.; around crests of ilia, 68 cm.; buttocks, greatest dimension, 85.5 cm.; thigh, right, 49.5 cm.; left, 48.5 cm.; calf, right, 31.5 cm.; left, 32.5 cm.; ankle, right, 19 cm.; left, 19 cm.

Bones and Joints.—The skeleton is normal. Radiographs of the head show the sella to be normal in size. There is no abnormality of the small or large joints.

Glands.—There is no enlargement of the lymph nodes. The thyroid gland is somewhat enlarged. The radiograph shows no evidence of the thymus.

Thorax.—Symmetrical. Expansion full and equal. The intercostal spaces are well marked. The clavicles, scapulæ, spinous processes of the vertebræ and muscles are prominent. The breasts are pendulous and the skin covering them devoid of subcutaneous fat. The lungs are negative on physical and roentgenographic examination. (Frederick H. Baetjer, roentgenographer.)

Cardiovascular.—The heart is normally situated. The sounds are clear at the apex and base. The aortic second sound is sharply accentuated. The radials and brachials are distinctly sclerosed. The pulse, with the patient in the recumbent posture, is 80 to the minute, regular in rate and rhythm. The systolic blood pressure is 204 and the diastolic, 84. At a subsequent examination the pulse and blood pressure were recorder five times at intervals of 15 minutes. The pulse ranged from 92 to 104 per minute, and the systolic pressure, from 212 to 220. The patient was somewhat excited at the time and because of her hypertension it was thought best to omit the adrenalin test.

The Blood.—The Wassermann blood test is negative; hæmoglobin, 82 per cent; leucocytes, 12,200; red blood cells, 5,284,000; smear, Wilson stain, slight anisocytosis and poikilocytosis; color index about 1; no parasites; platelets increased in size and markedly increased in number. There is a moderate leucocytosis with a slight increase in

* The method for measuring the folds of the skin as suggested by Oeder was used. A fold of skin is raised from the muscles by an assistant who holds it between the thumbs and index fingers, the grasping fingers being held about 3 or 4 cm. apart. This double layer of skin and subcutaneous fat is then measured with an ordinary mechanic's caliper. (G. Oeder, Med. Klin., 1910, VI, 657.)

Fig. 1.—Author's patient at the age of 12 years, two years before the onset of facial wasting.

Fig. 2.—Lipodystrophia progressiva. Author's patient, age 30 years. Front view. Notice the furrows produced by smiling.

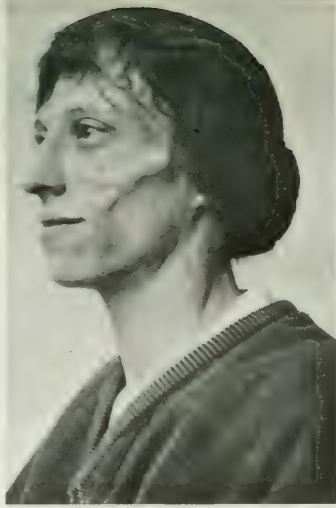

Fig. 3.—Side view of face.

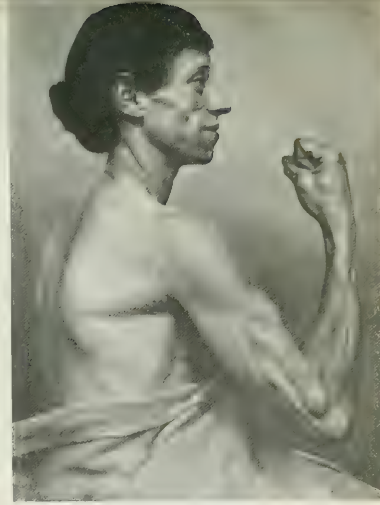

Fig. 4.—Side view showing facial wasting and prominence of muscles of upper extremity.

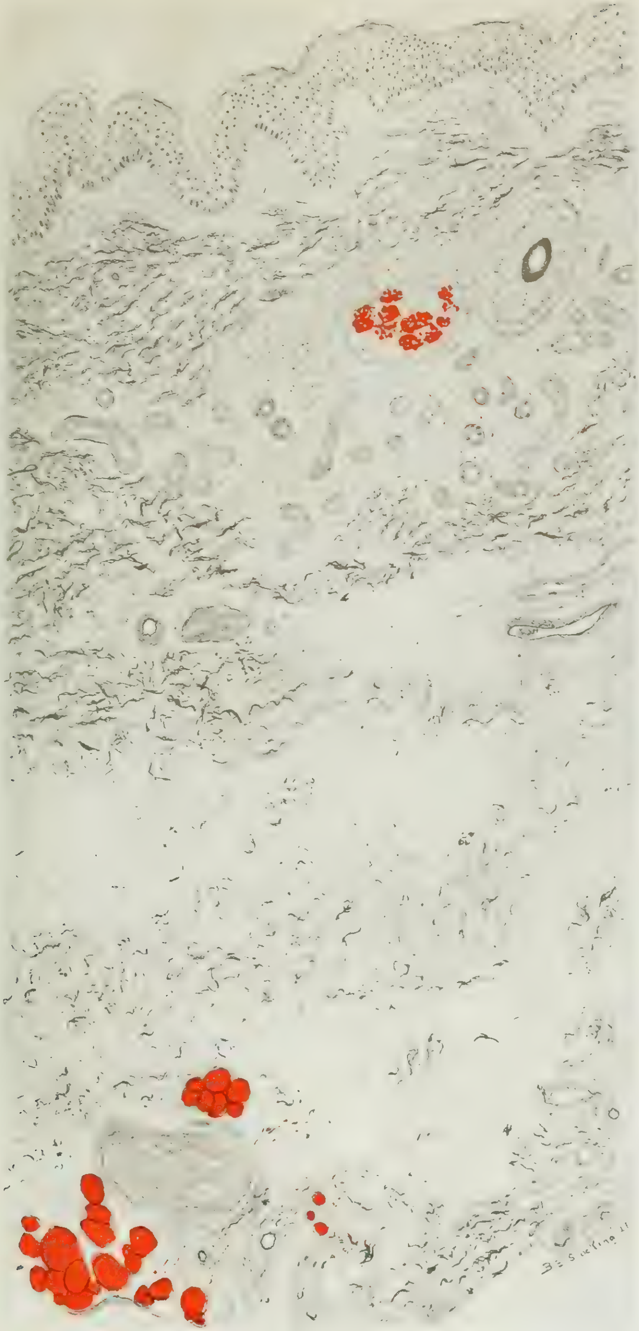

FIG. 9.—Microscopical section of a piece of skin, removed from left forearm of author's patient, showing corium and subcutaneous tissue with almost complete absence of subcutaneous fat. Fat stained with sudan.

FIG. 10.—Control illustration, after Simons showing abundance of subcutaneous fat in a section from the scalp of a healthy but not obese man, aged 34 years. Fat stained with sudan.

the polymorphonuclears. With the exception of moderate leucocytosis and the increase in the platelets, the blood picture is normal. (F. D. Conroy.)

Blood Chemistry.—The blood chemistry reveals a normal condition. The blood CO_2, 59.4 volumes per cent; the blood urea, 18.1 milligrams urea nitrogen per 100 c. c. (Walter W. Palmer.)

Blood Sugar.—The blood sugar curve is normal. (Leslie N. Gay.)

Abdomen.—The abdomen is scaphoid; walls, soft; no tenderness; no masses. The liver and spleen are not palpable. The double skin-fold of the abdomen, as stated, measures 4 mm.

Digestive System.—The color of the lips and mucous membrane is normal. The gums are in good condition. The teeth are sound; several molars are missing; one molar is capped. The tongue is clean and is protruded in the mid-line without tremor. Fluoroscopic examination reveals visceroptosis with normal stomach. Examination of the gastric contents after a rice test meal shows normal findings. The stool is normal in appearance. There are no ova; no occult blood.

Genito-Urinary.—There is no gynecological disease.

Urine.—Single specimen, clear, light straw; reaction, acid; sp. gr., 1014; heavy trace of albumin; no sugar; no casts. Twenty-four hour specimen, 995 c. c.; clear straw; acid, sp. gr., 1012; albumin present. Tests for sugar, diacetic and diazo-reaction and urobilin are negative. Microscopically, a few hyaline and granular casts; no red blood cells; an occasional white cell. Albumin and casts were present on several subsequent examinations.

Renal Function.—There is a phenolsulphonephthalein output of 21 per cent, first hour; second hour 12 per cent.

Basal Metabolism.—The metabolic rate shows a decided acceleration. The CO_2 output per square meter of body surface per hour is 36.8 per cent above the expected average for the patient's age and sex. (John T. King, Jr.)

Eye, Ear, Nose, Sinuses and Throat.—There is slight protrusion of the eyes. Right pupil is larger than the left. Both react promptly to light and accommodation. The eye movements are normal in all directions. No nystagmus. The convergence is normal, and except the slight exophthalmos and slight lid lag, there are no signs suggesting hyperthyroidism. The fundi show a low grade of optic neuritis and definite sclerosis of the retinal vessels. The ears, nose, accessory sinuses and throat are normal.

Neurological Examination.—The musculature of the entire body is normal in development, strength and function. There is no incoordination, no sign of muscular atrophy, no fibrillary twitching and no change in the electrical reactions. The fingers show a slight tremor.

The skin reveals no evidence of trophic change, but there is a marked abscence of subcutaneous fat in the face, neck, shoulders, arms, forearms, hands, chest, back, and abdomen and also, an increase of subcutaneous fat in the buttocks, thighs and legs.

The sensory and vasomotor functions are not abnormal.

The deep reflexes are active. The superficial reflexes are present with the exception of the abdominal skin reflex.

Romberg's, Trousseau's, Kernig's, and Babinski's signs are absent.

With the exception of moderate optic neuritis, the nerves of special sense are normal.

Mentality.—The patient is alert and very intelligent. (G. Lane Taneyhill.)

The case is one of lipodystrophia progressiva, associated with arteriosclerosis, chronic nephritis, hypertension, and a moderate thyropathy as shown by the slight enlargement of the thyroid gland, the rather prominent eyes, the slight lid lag, the tremor, the slight nervousness, the increase in the pulse rate, the acrocyanosis and the accelerated metabolic rate.

Summary

Lipodystrophia progressiva is a relatively rare condition, beginning insidiously and usually in early life; caused, possibly, by endocrine dysfunction; not hereditary; does not endanger life; and, is more commonly and more characteristically developed in the female. It is characterized pathologically by a slowly progressive, almost complete and probably permanent disappearance of the subcutaneous fat from the head, face, neck, upper extremities, and from the trunk as far as the pelvic bones and groin folds, and, especially in the female, by an increase in the subcutaneous fat of the buttocks, thighs and legs.

In conclusion I wish to express my thanks to Professor William G. MacCallum for the histological examination of the specimen of skin from my patient, and also for the excellent drawing of a microscopic section of the skin which accompanied the report. I am further indebted to Dr. G. Lane Taneyhill, Jr., for the neurological examination and for having pointed out the nature of the case, and to others for special examinations.

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AN EXPERIMENTAL STUDY OF PHAGOCYTOSIS IN RELATION TO TERMINAL INFECTIONS

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Introduction

The circumstances which originally suggested this investigation were the instances of energetic phagocytosis frequently observed at autopsy. The surprising number of bacteria occasionally found within the leucocytes in individuals dying of fatal infection or intoxication suggests the possibility that the diminution of phagocytic activity sometimes associated with destructive infections may not be as nearly universal as is commonly supposed. It further seems probable that in moribund animals, " in which the resistance fails,"[1] the phagocytic defense may remain unimpaired, and, occasionally at least, function at a level considerably above the normal. These observations also indicate that terminal infections cannot be accounted for by assuming a collapse or a decrease in the activity of the phagocytic functions of the animal.

Fig. 1 illustrates the extent of phagocytosis which may occur in animals dying of fatal infections.

Historical Sketch

The widespread occurrence of terminal infections was strikingly expressed by Osler when he wrote that, " Persons rarely die of the diseases with which they suffer."[2] However, the nature of the rupture in the defensive mechanism of the individual which permits the rapid invasion of the body before death by even the feebly aggressive bacteria, has never been satisfactorily explained. Flexner[3] undertook a study of this condition as early as 1896. He, however, limited his investigations to an examination of the humoral defense without any attempt at ascertaining whether cellular modifications might not be a contributing cause in terminal infections. He thought that he discovered, at least in a few cases, a slight decrease in the bactericidal actions of human serum against staphylococci. The correctness of these findings, however, was soon placed in doubt by Wright,[4] who, together with Windsor, demonstrated in 1902 that human blood exhibits an almost total absence of any bactericidal influence against staphylococci.

Bordet[5] was one of the first to suggest that the phagocytic activity of the body might be a relatively stable function and not easily influenced by conditions which profoundly affect other vital activities. He demonstrated that deep chloroform anesthesia which " completely deadens "[5] the central nervous system has no disturbing influence upon phagocytosis. This investigator also observed that diphtheria toxin exerts little, if any, effect on phagocytic activity.[6]

Tunnicliff,[7] in an investigation of the opsonic index during the leucopenia resulting from measles, found that there was a slight decrease for streptococci, staphylococci, and tubercle bacilli. She suggested that the decrease in phagocytic activity against these organisms might " account for the secondary infections " commonly associated with measles.

Bartlett and Ozaki,[8] in 1917, injected a dog with a massive dose of *B. coli* and five hours later, when the animal was in a moribund condition, inoculated it with a quantity of staphylococci. They observed no decrease in the opsonic index for staphylococcus. This experiment was not repeated and the authors themselves were not entirely convinced of the adequacy of their controls.

There is, of course, an extensive literature (Metchnikoff;[9] Wright;[10] Bull;[11] Hektoen;[12] Opie,[13] and others) indicating a sharp and very considerable decline, in some instances at least, in the phagocytic activity of the body against the specific infecting organism in fatal infections. There seems to have been no organized inquiry, however, concerning the opsonic index for those bacteria which take no part in the original infection but which later invade the weakened body giving rise to the destructive phenomenon known as terminal infection.

Bacterial invasion of the wasted body immediately preceding death is sometimes so complete and sudden that it has been assumed the process proceeds unopposed. The collapse of the defensive mechanism against bacteria in terminal infections seems to be thorough and complete. Zinsser[14] expressed this idea in a discussion of fatal infections when he wrote: " The infectious process becomes rapidly generalized, the bacteria enter the blood-stream and lymphatics, and the defensive powers are overwhelmed." The possibility of a distinct part

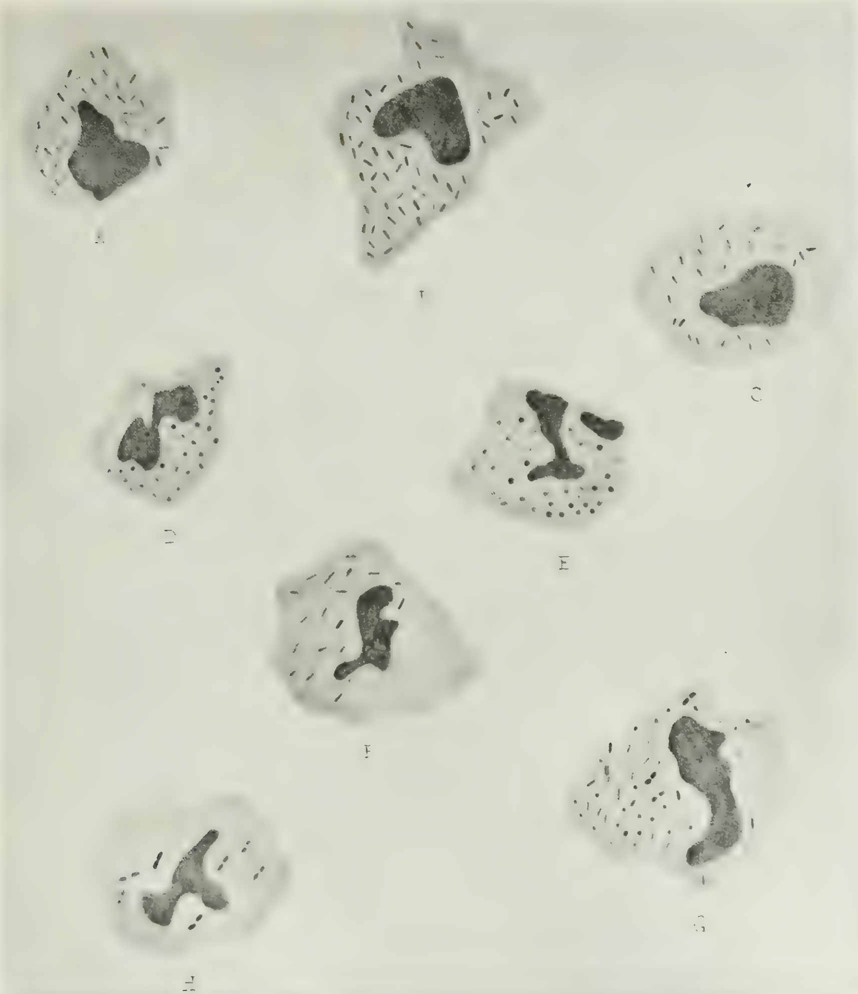

FIG. 1.—Illustrating the extent of phagocytosis which may occur during the late states of terminal infection. *A* and *B*. Cells from the peritoneal cavity of a guinea-pig dying of *B. coli* peritonitis. *C*. Phagocyte from lung of patient dying of influenza pneumonia. *D*. Leucocyte from peritoneal cavity of a guinea-pig dying of staphylococcus peritonitis. *E*. Leucocyte from blood stream of a guinea-pig dying of generalized staphylococcus infection. *F*. Phagocyte containing diphtheria bacilli from lung of child with terminal pneumonia following diphtheria. *G*. Cell containing mixed flora from lung of patient dying of terminal pneumonia. *H*. Phagocyte from pleural exudate of a boy dying of generalized pneumococcus infection.

of this protective adaptation of the body remaining intact, with unimpaired function, has not been believed and awaits confirmation. It is proposed, therefore, in this research to undertake an investigation of the phagocytic activity in animals after kataphylaxis * has occurred, and to determine what change, if any, occurs in the opsonic index for those organisms which most frequently overrun the body late in fatal infections and intoxications.

Plan of Investigation

The investigation of this problem has been done almost entirely *in vitro* because there is no thoroughly dependable method of controlling results when the experiments are undertaken *in vivo*. Wright's[15] work has established that even slight changes in the phagocytic activity of the animal can be demonstrated by *in vitro* methods, so that if there is a decline in opsonic power sufficient to account, even in part, for the bacterial invasion occurring in terminal infections, there could be no difficulty in demonstrating that decrease in effectiveness by the methods employed in this investigation. The bacteria used were those most commonly associated with terminal infections—a pyogenic coccus, *Staphylococcus aureus,* recently isolated from a case of human furunculosis, and a Gram-negative bacillus, *B. coli communis.* In addition, two other organisms were commonly used. *B. bronchisepticus* was selected because it is readily taken up in large numbers, making possible dependable counts in weakly phagocytic cells, while *B. typhosus,* resisting engulfment to considerable extent, permits reliable enumeration when phagocytosis with other bacteria is so vigorous that even a depressed phagocytic capacity is quite sufficient to fill the cells beyond counting. Whenever possible the specific infecting organism was included in each series of experiments.

Technique

The opsonic technique used in this research was essentially the same as that originally employed by Wright and Douglas.[16] Certain modifications and refinements, however, were introduced which seemed to insure greater uniformity in the results, and to render this generally confusing technique more dependable and satisfactory.

The blood providing the cells was received into a centrifuge tube containing several volumes of citrated salt solution. Before sedimentation the cells were uniformly suspended by repeated suction and ejection with a pipette. This process was repeated before each centrifugation and is a most effective means of preventing the aggregation of the platelets around the leucocytes. The centrifugation was accomplished at a speed which permitted the sedimentation of the white cells in four to five minutes without throwing down the platelets. This is most important, for it eliminates the platelets from the leucocytic layer and prevents packing of the white cells. The washings were repeated three times and the leucocytes suspended in a volume of physiological salt solution equal to one third the original volume of blood. After standing for one hour, cells were uniformly suspended by a gentle continued agitation of the tube and all samples of any one experimental series were taken immediately. It was early observed that the ingesting capacity of phagocytes was not always so reliable and vigorous if they were used after washing as when they were allowed to stand for an hour. This is probably occasioned by a disturbance in the osmotic pressure resulting from repeated washings and centrifugation.

Confusing and contradictory results invariably accompanied a careless or hurried preparation of the bacterial suspension. The organisms used in this work were grown, whenever possible, on moist plain agar for 15 hours. The tube was then washed out with saline to remove lint and debris. Five cubic centimeters of salt solution were next added and the tube gently agitated until the resulting turbidity was equal to or slightly in excess of that desired. This was then transferred to a second clean tube and vigorously shaken to break up possible clumps. Finally, the tube was centrifuged at a speed sufficient to remove from suspension all but the single bacteria. Two and five-tenths cubic centimeters were then drawn from the upper portion and reserved for the bacterial suspension. All the samples for any experimental series were regularly taken from the same level in order to insure a more nearly uniform number of organisms. Bacteria which do not grow upon plain agar or which could not be uniformly suspended from solid media were grown in meat infusion broth. For growing massive cultures of pneumococci, whole blood was added to the broth. The bacteria were sedimented, washed and suspended in salt solution. A turbidity was selected which presented a delicate opalescence in indirect light.

Sera were taken in the usual manner except that those containing an excessive amount of fat were, whenever possible, avoided. Those containing agglutinins for the experimental cells, although thoroughly annoying, can be used without invalidating the results. Although a difference of a few hours in the age of the sera results in no demonstrable difference in their opsonic reactions, all sera for an experimental series were collected as nearly as possible at the same time.

The phagocytic mixtures were prepared according to the Wright technique, and incubated at 37° C. for 20 minutes. The smears and stains were made in the manner described by Cross.[17] All smears showing gross differences in the number or distribution of the leucocytes were discarded and the preparations repeated. The organisms in 50 polymorphonuclear leucocytes were counted and these cells were always enumerated from corresponding areas on the control and experimental slides. Cells containing an excessively large number of bacteria show a tendency to collect in portions of the smear which can be predicted, and the most confusing and contradictory deviations present themselves if the selection of corresponding

* The word kataphylaxis was introduced by Bullock and Cramer (Proc. of the Royal Society, 1919, Series B, vol. 90, p. 513) and was defined by them as a rupture in the local defensive mechanism against bacteria. As used in the present paper, the word will be understood to indicate a break-down in the general defensive mechanism sufficiently complete to permit the infection to lead uninterruptedly to the death of the animal.

areas for enumeration is not rigorously observed. All preparations exhibiting marked variations from the normal or expected were repeated throughout. Whenever the phagocytes revealed unusual inequalities in the number of ingested organisms, parallel preparations were made and the average enumeration taken as the true count. Polymorphonuclear leucocytes alone were considered, and no attempt was made at enumeration in cells containing more than 25 organisms. In all counts the percentage of ingesting leucocytes as well as the total number of intracellular bacteria were determined.

Subject Matter

The data presented in this paper are selected from a study of 85 cases of infection which resulted in death. An effort was made to secure the widest possible variety, including both spontaneous and induced infections. The period elapsing between the primary inoculation and death ranged from a few hours to several weeks. The arrangement of subject matter and condition of each experiment are clearly set forth in the various protocols.

Experimental Data

PART I

A. *Pneumococcus Infections*

Experiment 1.—The dog, No. 11, used in this experiment was an adult female weighing 15½ pounds. It was inoculated intravenously on November 27, 1920, with a sublethal dose of virulent pneumococcus, Type I. The sublethal doses were continued on dates indicated in the protocol until the blood exhibited a high bacteriotropic action. The intravenous inoculations were then gradually increased until a sufficient dose was administered to bring about the death of the animal. This result was hastened toward the end of the experiment by the injection of pneumococci into the left pleural cavity. A sample of blood was taken before each injection and allowed to clot. Two hours later the serum was withdrawn and the opsonic index determined in the manner described above. The normal dogs providing the leucocytes and control serum were always bled at the same time as the experimental animal. The results of all opsonic determinations are included in the following tables:

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
11-27-20	Dog No. 11. Before injection wt. 15½ lbs. Inj. 25 c. c. Pn. I.	S. aureus....	No. 11	50	39	11	145	2.9*
			Con.	50	42	8	121	2.4
		Pn. I........	No. 11	50	1	49	2	0.0
			Con.	50	4	46	7	0.1
		B. coli	No. 11	50	41	9	86	1.7
			Con.	50	40	10	93	1.8
		B. typhosus.	No. 11	50	43	7	95	1.9
			Con.	50	45	5	104	2.0
11-29-20	Dog No. 11. Wt. 14½ lbs. Inj. 50 c. c. Pn. I.	S. aureus....	No. 11	50	42	8	175	2.5
			Con.	50	43	7	171	3.4
		Pn. I........	No. 11	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	43	7	96	1.7
			Con.	50	46	4	104	2.0
		B. typhosus.	No. 11	50	35	15	66	1.3
			Con.	50	38	12	70	1.4
12-1-20	Dog No. 11. Wt. 13½ lbs. Inj. 100 c. c. Pn. I.	S. aureus	No. 11	50	41	9	220	4.4
			Con.	50	44	6	212	4.2
		Pn. I........	No. 11	50	26	24	101	2.0
			Con.	50	5	45	6	0.1
		B. coli	No. 11	50	19	31	50	1.0
			Con.	50	25	25	55	1.1
		B. typhosus.	No. 11	50	13	37	21	0.4
			Con.	50	15	35	20	0.4
12-3-20	Dog No. 11. Wt. 14 lbs. †Inj. 150 c. c. Pn. I.	S. aureus....	No. 11	50	24	26	57	1.1
			Con.	50	27	23	60	1.2
		Pn. I........	No. 11	50	31	19	100	2.0
			Con.	50	2	48	4	0.1
		B. coli	No. 11	50	23	27	28	0.5
			Con.	50	24	26	35	0.7
		B. typhosus.	No. 11	50	39	11	82	1.6
			Con.	50	42	8	85	1.6
12-10-20	Dog No. 11. Wt. 13 lbs. Inj. 200 c. c. Pn. I.	S. aureus....	No. 11	50	41	9	106	3.3
			Con.	50	38	12	145	2.9
		Pn. I........	No. 11	50	38	12	162	3.2
			Con.	50	8	42	22	0.4
		B. typhosus.	No. 11	50	21	29	30	0.6
			Con.	50	20	30	45	0.9
12-17-20	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	S. aureus....	No. 11	50	40	10	269	3.3
			Con.	50	43	7	231	4.6
		Pn. I........	No. 11	50	25	25	75	1.5
			Con.	50	10	40	21	0.4
		B. coli	No. 11	50	32	18	77	1.5
			Con.	50	33	17	80	1.6
		B. typhosus.	No. 11	50	32	16	70	1.4
			Con.	50	38	12	86	1.7
12-22-20	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	S. aureus....	No. 11	50	33	17	121	2.4
			Coh.	50	25	25	104	2.0
		Pn. I........	No. 11	50	25	25	161	3.2
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	43	7	113	2.2
			Con.	50	38	12	105	2.1
		B. typhosus.	No. 11	50	30	20	50	1.0
			Con.	50	31	19	68	1.3
12-26-20	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	....	......	....	....	....	....	....
12-30-20	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	....	..	....	....	....	....	....
1-10-21	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	....	......	....	....	....	....	....
1-10-21	Dog No. 11. Wt. 13 lbs. Inj. 250 c. c. Pn. I.	S. aureus....	No. 11	50	37	13	199	3.9
			Con.	50	33	17	180	3.6
		Pn. I........	No. 11	50	22	28	62	1.3
			Con.	50	8	42	16	0.3
		B. coli	No. 11	50	41	9	103	2.0
			Con.	50	45	5	140	2.8
		B. typhosus.	No. 11	50	26	24	27	0.5
			Con.	50	23	27	38	0.7
1-20-21	Dog No. 11. Wt. 13 lbs. ‡Inj. 245 c. c. Pn. I.	S. aureus....	No. 11	50	36	14	146	2.9
			Con.	50	35	15	171	3.4
		Pn. I........	No. 11	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	42	8	93	1.9
			Con.	50	41	9	95	1.9
		B. bronchi-sept.	No. 11	50	27	23	65	1.3
			Con.	50	42	8	73	1.4
1-22-21	‡Inj. 50 c. c. Pn. I.	....	......	....	....	....	....	....
1-24-21	Dog No. 11. Wt. 11 lbs. No inj.	S. aureus...	No. 11	50	34	16	128	2.5
			Con.	50	31	19	115	2.3
		Pn. I........	No. 11	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	41	9	78	1.5
			Con.	50	41	9	71	1.4
		B. bronchi-sept.	No. 11	50	37	13	136	2.7
			Con.	50	38	12	137	2.7
1-25-21	Dog No. 11. Wt. 11 lbs. This reading made 10 hours before death. No injection.	S. aureus....	No. 11	50	35	15	235	4.7
			Con.	50	32	18	199	3.9
		Pn. I........	No. 11	50	4	46	8	0.1
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	15	35	45	0.9
			Con.	50	14	36	50	1.0
		B. bronchi-sept.	No. 11	50	39	11	212	4.2
			Con.	50	37	13	196	3.9
		B. typhosus.	No. 11	50	7	43	15	0.3
			Con.	50	8	42	19	0.3
1-25-21	Dog No. 11. Wt. 11 lbs. This reading made at the time of death.	S. aureus....	No. 11	50	34	16	251	5.0
			Con.	50	33	17	199	3.9
		Pn. I........	No. 11	50	7	43	15	0.3
			Con.	50	0	50	0	0.0
		B. coli	No. 11	50	14	37	47	0.9
			Con.	50	14	36	50	1.0
		B. bronchi-sept.	No. 11	50	33	17	226	4.5
			Con.	50	37	13	195	3.9
		B. typhosus	No. 11	50	6	44	16	0.3
			Con.	50	8	42	19	0.3

* The opsonic indices which are plotted in the accompanying graphs are obtained for each microorganism by dividing the phagocytic index of the experimental animal for this bacterium by the corresponding phagocytic index of a normal control animal.

† Pneumococci were grown in meat infusion broth containing 1% whole blood. The bacteria for each injection were sedimented from the quantity indicated in the table, and then suspended in 25 c. c. of broth before inoculation.

‡ The bacteria from 25 c. c. of this inoculation were injected into the left pleural cavity. The remaining quantity was given intracardially.

Anatomical Diagnosis.—Slight emaciation, broncho-pneumonia, purulent exudate in both pleural cavities, generalized edema. Pneumococcus, Type I, cultivated in pure culture from pleural exudate and from heart's blood.

Kataphylaxis in this animal probably occurred on January 20, five days before its death, and was first indicated by the appearance of pneumococci in the blood in sufficient quantity to be recovered in the culture. By referring to the curves in Fig. 2 it will be observed that there was no post-kataphylactic

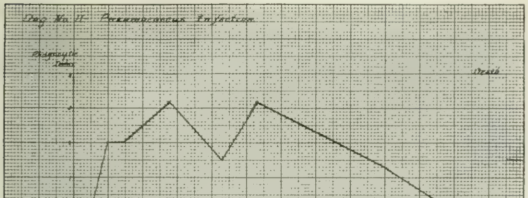

FIG. 2.

decline in the opsonic index for any except the specific infecting organism. Samples of blood taken a few moments before death, when the animal was in a state of complete collapse, revealed a phagocytic activity in all respects equal to the normal and for staphylococci it was even somewhat increased.

The opsonic index for the infecting organism behaved throughout in accordance with the findings published by Neufeld and Töpfer[18] and Rosenow.[19] Virulent pneumococci are not phagocyted at all by the normal animal, but after one or two inoculations of this organism the opsonins for pneumococci increase and can be still further increased by subsequent injections, provided a lethal dose is not administered. Once this quantity is given, however, there is a rapid decline in phagocytic activity, the zero mark ordinarily being reached some time before the death of the animal.

The variation in the opsonic curve for *Staphylococcus aureus* requires some explanation. Up to December 3 the curve for this organism had remained normal, but at this time a culture of Pneumococcus I, contaminated with *Staphylococcus aureus* was injected and five days later the dog's blood showed an increase in opsonins for staphylococci. This organism was almost immediately overcome, for it was never obtained in subsequent blood cultures. The increased opsonic activity for staphylococci continued a few days, but gradually decreased until at the end of a month the index was again normal. However, on January 20 pneumococci appeared in the blood and the reaction against this homologous antigen resulted in a non-specific increase in the residue of antibodies remaining from the previous staphylococcus inoculation. This was immediatelyy reflected by a rise in the opsonic curve for Staphylococcus. These observations are in agreement with the findings of Bieling.[20]

B. *Staphylococcus Infections*

EXPERIMENT 2.—An adult female dog, No. 14, weighing 15 pounds was selected as the experimental animal in this test. She was given an initial intravenous injection of 1 c.c. of a broth culture of *Staph. aureus* on November 27, 1920. Gradually increasing amounts of this organism were given until the dog, in an emaciated and helpless condition, died 23 days later.

The opsonic determinations, together with other data concerning this experiment are recorded in the appended table.

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
11-7-20	Dog No. 14. Wt. before injunction 15 lbs. Inj. 1 c. c. S. aureus.	S. aureus....	No. 14	50	41	9	121	2.4
			Con.	50	42	8	120	2.4
		Pn. I........	No. 14	50	5	45	10	0.2
			Con.	50	4	46	8	0.1
		B. typhosus.	No. 14	50	42	8	106	2.1
			Con.	50	45	5	104	2.0
11-29-20	Dog No. 14. Wt. 13½ lbs. Inj. 2 c. c. S. aureus.	S. aureus....	No. 14	50	44	6	267	5.3
			Can.	50	44	6	170	3.4
		Pn. I........	No. 14	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli......	No. 14	50	38	12	96	1.9
			Con.	50	46	4	104	2.0
		B. typhosus.	No. 14	50	35	15	100	2.0
			Con.	50	38	12	70	1.4
12-1-20	Dog No. 14. Wt. 13½ lbs. No inj.	S. aureus....	No. 14	50	50	0	309	6.2
			Con.	50	44	6	212	4.2
		Pn. I........	No. 14	50	4	46	10	0.2
			Con.	50	5	45	6	0.1
		B. coli......	No. 14	50	34	16	56	1.1
			Con.	50	35	15	55	1.1
		B. typhosus	No. 14	50	14	36	18	0.3
			Con.	50	15	35	20	0.4
12-3-20	Dog No. 14. Wt. 13 lbs. Inj. 5 c. c. S. aureus.	S. aureus....	No. 14	50	28	22	66	1.3
			Con	50	27	23	61	1.2
		Pn. I........	No. 14	50	3	47	7	0.1
			Con.	50	2	48	4	0.0
		B. coli	No. 14	50	31	19	50	1.0
			Con.	50	34	16	35	0.7
		B. typhosus.	No. 14	50	36	14	76	1.5
			Con.	50	42	8	85	1.6
12-7-20	Dog No. 14. Wt. 12½ lbs. No Inj.	S. aureus....	No. 14	50	35	15	73	1.5
			Con.	50	34	16	150	3.0
		Pn. I........	No. 14	50	....	....	....	....
			Con.	...	....	....	....	....
		B. coli......	No. 14	50	49	1	156	3.1
			Con.	50	39	11	131	2.6
		B. typhosus.	No. 14	....	....	....	....	....
			Con.	....	....	....	....	....
12-10-20	Dog No. 14. Wt. 11 lbs. No inj.	S. aureus....	No. 14	50	41	9	76	1.5
			Con	50	39	11	115	2.3
		Pn. I........	No. 14	50	....	....	....	....
			Con.	50	....	....	....	....
		B. coli......	No. 14	50	31	19	73	1.5
			Con.	50	33	17	75	1.5
		B. typhosus.	No. 14	50	19	31	42	0.8
			Con	50	23	27	33	0.6

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
12 15 20	Dog No. 14. Wt. 11 lbs. No. inj.	S. aureus	No. 14	50	29	21	105	2.1
			Con.	50	25	25	171	3.4
		Pn. I	No. 14	50	....	....	....	....
			Con.	50	....	....	....	....
		B. coli	No. 14	50	38	12	87	1.7
			Con.	50	42	8	73	1.5
		B. typhosus	No. 14	50	27	24	55	1.1
			Con.	50	28	22	46	0.9
12 17 20	Dog No. 14. No inj.	S. aureus	No. 14	50	44	6	354	7.0
			Con.	50	43	7	231	4.6
		Pn. I	No. 14	50	9	41	22	0.4
			Con.	50	10	40	21	0.4
		B. coli	No. 14	50	35	15	71	1.4
			Con.	50	33	17	80	1.6
		B. typhosus	No. 14	50	40	10	92	1.8
			Con.	50	38	12	86	1.7
12 19 20 12.00 P. M.	Dog No. 14. Reading made at time of death	S. aureus	No. 14	50	36	14	121	2.4
			Con.	50	38	12	219	4.4
		Pn. I	No. 14	50	4	46	18	0.3
			Con.	50	5	45	12	0.2
		B. coli	No. 14	50	41	9	111	2.2
			Con.	50	42	8	91	1.8
		B. typhosus	No. 14	50	29	[illegible]	72	1.4
			Con.	50	30	20	49	1.0

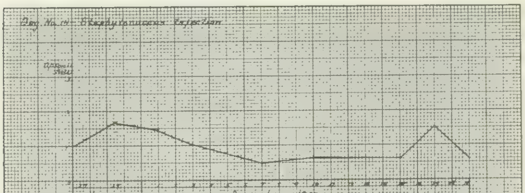

FIG. 3

Anatomical Diagnosis.—Marked emaciation, Staphylococcus broncho-pneumonia, multiple abscesses in body wall, heart, kidneys and spleen, acute nephritis, staphylococcus cystitis. *Staph. aureus* in pure culture obtained from heart's blood, kidneys, spleen and urine.

The variations in the opsonic index for *Staph. aureus* in this experiment are in agreement with the results published by Wright[21] and Neufeld.[22] Under the stimulation of sublethal inoculation there is an increase in phagocytic activity but as soon as the lethal dose is given there is a sharp decline which often forces the index below the normal. One especially interesting feature of the opsonic index for Staphylococcus in this experiment is that on December 7th the index was somewhat lower than it was at the time the animal died. Although phagocytosis is regarded as the chief defensive mechanism opposed against staphylococci, this animal with an uncomplicated infection survived the period of its lowest phagocytic activity and died when its opsonic index was only slightly lower than the normal.

This case represents an infection of the most violent and destructive nature. The animal lost 26 per cent of its body weight, and during the last 36 hours of life was in a state of complete exhaustion and collapse. Suppurative lesions occurred generally throughout the body. In spite, however, of the overwhelming character of this infection there was no decrease in phagocytic activity, even in the hours immediately preceding death, against any organisms not concerned in the primary infection.

C. *Typhoid Infections*

EXPERIMENT 3.—In this experiment an adult male dog, No. 13, weighing 13½ pounds, was injected intravenously on November 27, 1920, with 2 c.c. of an 18-hour broth culture of *B. typhosus*. Sublethal inoculations were continued until the blood revealed a high opsonic content and then the injections were gradually increased until the dog died on December 28, 1920.

Samples of blood were taken before each injection and the usual opsonic determinations made under the conditions described in the following table:

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
11 27 20	Dog No. 13. Wt. before injection 13½ lbs. Inj. 2 c. c. B. typhosus.	S. aureus	No. 13	50	48	2	133	2.6
			Con.	50	42	8	121	2.4
		Pn. I	No. 13	50	3	47	4	0.0
			Con.	50	4	46	8	0.1
		B. coli	No. 13	50	..	..	..	..
			Con.	50	..	..	..	..
		B. typhosus	No. 13	50	42	8	97	1.9
			Con.	50	45	5	104	2.0
11 29 20	Dog No. 13. Wt. 13½ lbs. No inj.	S. aureus	No. 13	50	47	3	150	3.0
			Con.	50	44	6	170	3.4
		Pn. I	No. 13	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 13	50	41	9	83	1.6
			Con.	50	46	4	102	[illegible]
		B. typhosus	No. 13	50	43	7	95	1.9
			Con.	50	[illegible]	11	71	1.4
12 1 20	Dog No. 13. Wt. 13½ lbs. Inj. 1 c. c. B. typhosus.	S. aureus	No. 13	50	43	7	218	4.3
			Con.	50	45	5	212	4.2
		Pn. I	No. 13	50	4	46	8	0.1
			Con.	50	4	46	6	0.1
		B. coli	No. 13	50	32	18	53	1.0
			Con.	50	25	25	55	1.1
		B. typhosus	No. 13	50	17	33	45	0.9
			Con.	50	15	35	20	0.4
12 3 20	Dog No. 13. Wt. 13 lbs. Inj. 4 c. c. B. typhosus.	S. aureus	No. 13	50	23	27	70	1.4
			Con.	50	27	23	63	1.2
		Pn. I	No. 13	50	4	46	8	0.1
			Con.	50	2	48	4	0.0
		B. coli	No. 13	50	24	26	37	0.7
			Con.	50	24	26	35	0.7
		B. typhosus	No. 13	50	49	1	300	6.4
			Con.	50	42	8	85	1.7
12 17-20	Dog No. 13. Wt. 11 lbs. Inj. 5 c. c. B. typhosus.	S. aureus	No. 13	50	46	4	232	4.6
			Con.	50	43	7	231	4.6
		Pn. I	No. 13	50	7	43	25	0.5
			Con.	50	10	40	21	0.4
		B. coli	No. 13	50	40	10	84	1.6
			Con.	50	33	17	80	1.6
		B. typhosus	No. 13	50	42	8	211	4.2
			Con.	50	38	12	86	1.7

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
12-22-20	Dog No. 13. Wt. 11½ lbs. Inj. 5 c. c. B. typhosus.	S. aureus....	No. 13	50	26	24	119	2.3
			Con.	50	25	25	104	2.1
		Pn. I........	No. 13	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 13	50	41	9	130	2.6
			Con.	50	38	12	105	2.1
		B. typhosus.	No. 13	50	40	10	211	4.2
			Con.	50	31	19	68	1.3
12-26-20 9:00 A. M.	Dog No. 13. Wt. 9½ lbs. No inj.	S. aureus....	No. 13	50	45	5	256	5.1
			Con.	50	41	9	250	5.0
		Pn. I	No. 13	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 13	50	25	25	43	0.8
			Con	50	37	37	47	0.9
		B. typhosus.	No. 13	50	27	27	77	1.5
			Con.	50	4	4	10	0.2
12-26-20 5:00 P. M.	Dog No. 13. Wt. 9 lbs. No inj.	S. aureus	No. 13	50	35	15	227	4.5
			Con.	50	41	9	250	5.0
		Pn. I........	No. 13	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 13	50	21	29	42	0.8
			Con.	50	37	13	47	0.9
		B. typhosus	No. 13	50	36	14	78	1.5
			Con.	50	4	46	10	0.2
12-28-20	Dog No. 13. 3 hrs. before death	S. aureus....	No. 13	50	23	27	146	2.9
			Con.	50	37	13	180	3.6
		Pn. I........	No. 13	50	2	48	2	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 13	50	36	14	62	1.2
			Con.	50	36	14	[illegible]	1.0
		B. typhosus	No. 13	50	32	18	50	1.0
			Con.	50	19	31	26	0.5
12-28-20	Dog No. 13. Wt. 8 lbs. Death.	S. aureus....	No. 13	50	27	23	143	2.8
			Con.	50	37	13	180	3.6
		Pn. I	No. 13	50	0	50	0	0.0
			Con.	50	0	50	0	0.0
		B. coli	No. 13	50	24	26	58	1.1
			Con.	50	26	16	[illegible]	1.0
		B. typhosus.	No. 13	50	38	12	70	1.4
			Con.	50	19	31	26	0.5

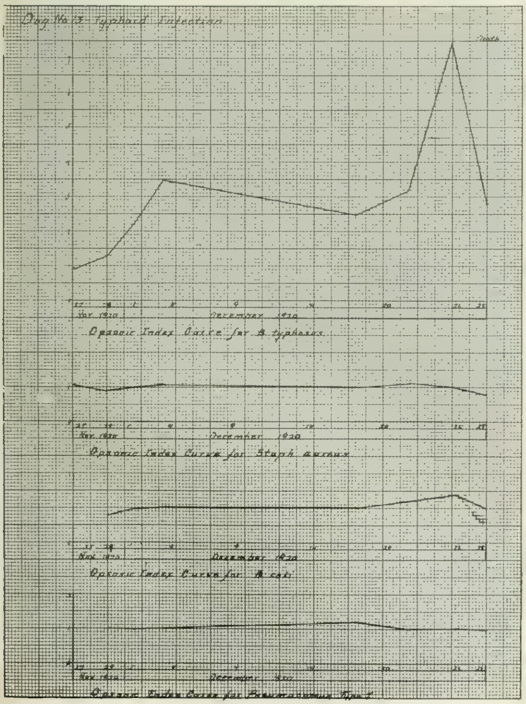

Fig. 4.

Anatomical Diagnosis.—Emaciated; no characteristic typhoid lesions except acute splenic tumor. *B. typhosus* obtained from heart's blood. *B. enteriditis* cultivated from heart's blood and gall bladder.

Kataphylaxis in this animal probably occurred on December 26th when there was a marked decline in the opsonic index for the typhoid bacilli. The decrease, however, was not sufficient to bring the index down to the normal. The phagocytic activity of the blood against *B. typhosus* was at the time of death more than twice as great as that of a normal control. It is possible, too, that the test gives an inadequate measure of the real opsonic strength of the experimental animal's blood, for Harrison[23] has shown that the lytic action of antityphoid serum is so great against the typhoid bacilli as to lower the observed index by dissolving the bacteria before they can be taken up by the leucocytes. That this condition actually existed in this case was demonstrated when the inactivated immune serum gave a higher phagocytic index than when the serum was used unheated. The results of this test are given in the following table:

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
12-18-20	Unhtd. serum.....	B. typho...	No. 13	50	38	12	70	1.4
	Inactvd. serum....		No. 13	50	42	8	124	2.4

That the phagocytic cells in this dog, dying of a typhoid infection, were not functionally impaired is indicated by the following tests.

A quantity of blood was taken in citrate from dog No. 13 and from a control animal. Each lot was washed twice, using 175 volumes of salt solution. Samples from the resulting cells were then separately incubated with *Staph. aureus* and *B. typhosus*. The results are given in the table.

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
12-28-20	30 min. incubation.	S. aureus....	No. 13	50	1	49	4	0.0
			Con.	50	2	48	2	0.0
	30 min. incubation.	B. typhosus.	No. 13	50	32	18	57	1.1
			Con.	50	0	50	0	0.0

Whether the energetic phagocytosis of *B. typhosus* by the washed cells of the immune animal, in the absence of serum, can be interpreted as the action of an immune cell, or whether there was left upon the cell enough highly potent immune serum to opsonize only the specific bacteria is a matter to be decided by more detailed observations. That the last explanation, however, is probably the true one is suggested by Klein's[24] work on the dilution of sera.

A quantity of polymorphonuclear leucocytes were recovered from the urine of dog No. 13 and when incubated with sensitized bacteria were actively phagocytic, as indicated in the following results:

Date	Remarks	Bacteria	*Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
12-28-20	Cells from urine of dog No. 13.	B. typhosus.	No. 8	50	33	17	128	2.6
			Con.	50	23	27	37	0.7

* Animal providing serum.

These findings, then, reveal an animal dying of an infection with its phagocytic mechanism, so far at least as experimental standards can determine, functioning with an efficiency considerably above the normal against the specific organism, without any demonstrable decrease in opsonic activity against the other bacteria used in the test.

Experiment 4.—In this experiment are presented the results of a study of four cases of fatal spontaneous hemolytic streptococcus infection in cats. The epidemic, of which these cases were a part, appeared in a room containing 25 cats, and ran a course so severe that of the whole number only two survived. The onset of the attack was characterized by a nasal discharge and violent sneezing. The cats lost rapidly in weight and died, generally after four or five days. The last 24 hours of the infection were especially severe. The animal was in a state of extreme emaciation and exhaustion, being all the time unable to stand and in the last hours of life responding only to such violent stimulation as a cardiac puncture.

Samples of blood were taken from the heart at intervals indicated in the tables and the usual opsonic determinations made.

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
2-13-21	Cat No. 33. At time of death.	S. aureus	No. 33	50	43	7	210	4.2
			Con.	50	46	4	188	3.7
		B. coli	No. 33	50	29	21	51	1.0
			Con.	50	27	23	40	0.8
		B. bronchisep.	No. 33	50	50	0	380	7.6
			Con.	50	46	4	285	5.7
		Strep. hemo.	No. 33	50	25	25	101	2.1
			Con.	50	32	18	266	5.3
2-14-21	Cat No. 35. One hr. before death.	S. aureus	No. 35	50	38	12	161	3.2
			Con.	50	38	12	137	2.7
		B. coli	No. 35	50	27	23	55	1.1
			Con.	50	25	25	62	1.2
		B. bronchisep.	No. 35	50	37	13	166	3.3
			Con.	50	34	16	172	3.4
		B. typhosus.	No. 35	50	36	14	71	1.4
			Con.	50	33	17	86	1.7
2-14-21	Cat No. 36. At time of death.	S. aureus	No. 36	50	37	13	138	2.7
			Con.	50	38	12	137	2.7
		B. coli	No. 36	50	23	27	58	1.1
			Con.	50	25	25	62	1.2
		B. bronchisep.	No. 36	50	43	7	216	4.3
			Con.	50	34	16	171	3.4
		B. typhosus.	No. 36	50	25	25	55	1.1
			Con.	50	33	12	86	1.7
2-15-21	Cat No. 37	S. aureus	No. 37	50	37	13	102	2.0
			Con.	50	50	0	256	5.1
		B. coli	No. 37	50	48	2	225	4.5
			Con.	50	48	2	216	4.3
		B. bronchisep.	No. 37	50	50	0	294	5.8
			Con.	50	42	8	250	5.0
		Strep hemo.	No. 37	50	25	25	146	2.9
			Con.	50	42	8	213	4.3
2-17-21	Cat No. 37. 28 hours before death.	S. aureus	No. 37	50	39	11	132	2.6
			Con.	50	46	4	188	3.7
		B. coli	No. 37	50	25	25	45	0.9
			Con.	50	18	32	42	0.8
		B. bronchisep.	No. 37	50	46	4	263	5.2
			Con.	50	48	2	284	5.6
		Strep. hemo.	No. 37	50	34	16	176	3.5
			Con.	50	36	14	266	5.3
2-19-21	Cat No. 37. At time of death.	S. aureus	No. 37	50	41	9	305	6.1
			Con.	50	46	4	340	6.8
		B. coli	No. 37	50	42	8	216	4.3
			Con.	50	29	21	159	3.2
		B. bronchisep.	No. 37	50	50	0	720	14.4
			Con.	50	50	0	793	15.9
		Strep. hemo.	No. 37	50	32	18	160	3.2
			Con.	50	41	9	201	4.0
2-19-21	Cat No. 37. One hr. after death.	S. aureus	No. 37	50	46	4	335	6.7
			Con.	50	48	2	327	6.5
		B. coli	No. 37	50	50	0	150	3.0
			Con.	50	45	5	120	2.4
		B. bronchisep.	No. 37	50	50	0	304	6.0
			Con.	50	50	0	297	5.9
		Strep. hemo.	No. 37	50	50	0	201	4.0
			Con.	50	50	0	386	7.7

Anatomical Diagnosis.—All the cats in this experiment presented, in general, the same appearance: Marked emaciation; greenish purulent exudate filling the nasal sinuses and extending over the surfaces of the nasopharynx and down into the trachea as far as the bifurcation. There was a complete absence of any lesions in any other organs. No hemorrhages. *Streptococcus hæmolyticus* in pure culture obtained from the heart's blood of all four animals. This was also the predominating organism in the exudate of the upper respiratory tract.

There is, in general, agreement in the results obtained from these four cases. In all the opsonic index for the specific organism was below normal at the time of death. The opsonic indices for the heterologous bacteria, in each instance, differed from the normal by less than what can properly be regarded as experimental error.

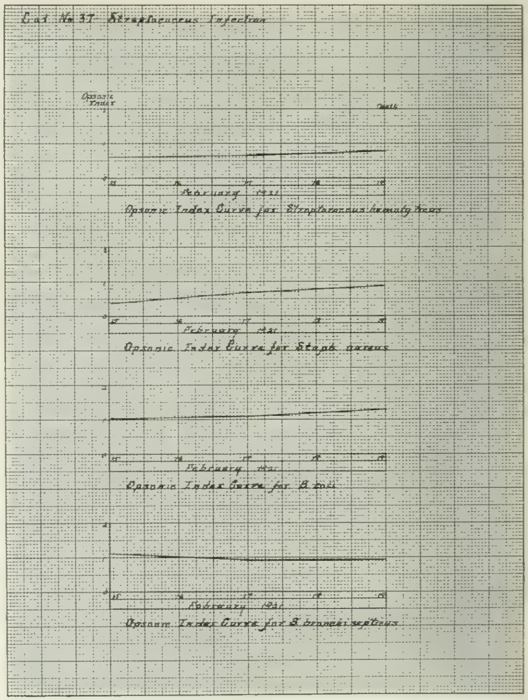

Fig. 5.

There was in the study of cat No. 37 one feature of very special significance and interest. This animal when first examined on February 15th already revealed advanced emaciation and weakness. The blood culture on this date showed an abundance of streptococci and a few colonies of *Staph. aureus.* Two days later this latter organism was found abundantly in the blood, but by February 19th it had considerably decreased and had completely disappeared before the cat died on February 20th. Ten cubic centimeters of blood cultured at the time of autopsy failed to develop a single colony of *Staphylococcus.* Streptococci were sufficiently numerous in the blood at all times to be recovered by plating a single loop of blood.

When this cat was placed under observation it was the subject of a double infection and showed indications of being rapidly overcome. That the streptococcus was the dominant etiological factor in the infection seems evident from the fact that the symptoms revealed by this cat were in every way similar to those in animals suffering with a pure streptococcus infection.

Cat No. 37, then, was being overcome by a rapidly fatal infection. It had in some way become secondarily infected with *Staphylococcus aureus,* and this latter organism had become so abundant in the blood stream that there resulted a marked decrease in the opsonins for staphylococci. Yet this enfeebled animal in its losing struggle against a streptococcus infection was able to react against a secondary infection of staphylococcus in a manner so vigorous as to banish completely these bacteria from the body. There was, during this time, a gradual increase in the opsonic index for staphylococcus until at the time of death it was only slightly below the normal. This increase is given in the following table:

		Date		
		Feb. 15	Feb. 17	Feb. 19
Cat No. 37.........	Op. ind.......	0.40	0.70	0.89

It would seem then that the body reacted against the secondary infection in an independent manner, and that recovery from one infection and death from another occurred, by chance, at the same time.

EXPERIMENT 5.—In this experiment an attempt was made to reproduce experimentally the spontaneous infections described in Experiment 4. An adult female cat, No. 38, was injected intravenously with 3 c. c. of an 18-hour culture made directly from the heart's blood of a cat dying of the epidemic streptococcus infection. The cat came down with the characteristic symptoms and died after 72 hours. The extent of emaciation in this animal was not as marked as in the preceding cases.

The routine determinations are recorded in the accompanying table.

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
2-18-21	Cat No. 38. Before injection. Inj. 3 c. c. Strep. hemo.	S. aureus....	No. 38	50	40	10	167	3.3
			Con.	50	46	4	188	3.7
		B. coli	No. 38	50	29	21	50	1.0
			Con.	50	18	32	42	0.8
		B. bronchi-sept.	No. 38	50	47	3	301	6.1
			Con.	50	48	2	284	5.6
		Strep. hemo.	No. 38	50	35	15	306	6.1
			Coh.	50	36	14	266	5.3
2-20-21	Cat No. 38. No injection.	S. aureus....	No. 38	50	39	11	400	8.0
			Con.	50	46	4	341	6.8
		B. coli	No. 38	50	45	5	155	3.1
			Con.	50	28	22	159	3.2
		B. bronchi-sept.	No. 38	50	50	0	850	17.0
			Con.	50	50	0	790	16.0
		Strep. hemo.	No. 38	50	29	21	191	3.8
			Con.	50	42	8	202	4.0
2-21-21	Cat No. 38. Death.	S. aureus....	No. 38	50	40	10	313	6.2
			Con.	50	47	3	328	6.6
		B. coli	No. 38	50	39	11	59	1.2
			Con.	50	45	5	120	2.4
		B. bronchi-sept.	No. 38	50	50	0	320	6.4
			Con.	50	50	0	297	5.9
		Strep. hemo.	No. 38	50	33	17	224	4.5
			Con.	50	20	30	286	7.7

Anatomical Diagnosis.—Slight emaciation; *Streptococcus hæmolyticus* in pure culture cultivated from the heart's blood.

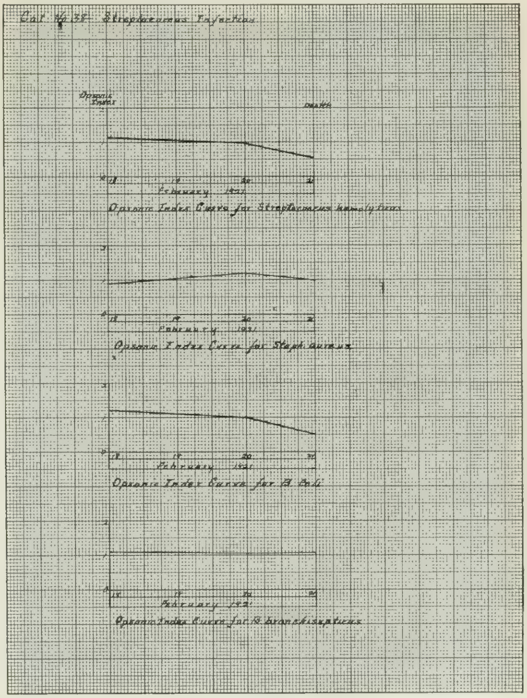

FIG. 6.

The findings in this experiment are throughout similar to the results obtained in the spontaneous streptococci infections.

EXPERIMENT 6.—The dog studied in this experiment had, when first seen on December 27, already developed characteristic symptoms of "distemper." There was a continual nasal discharge associated with frequent sneezing and dyspnea. The animal was emaciated and staggered about when forced to its feet. A small Gram-negative bacillus, probably *Pasteurella canis,* was present in enormous numbers in both the nasal secretion and blood stream. However, on December 30 the dog began to improve and continued to gain in strength until January 12. On this date it suffered an overwhelming and paralyzing collapse. After this, the dog was unable to move and lay in a continual stupor. It died on January 14, in a state of weakness so profound as at once to set it apart from any other animal observed during this investigation.

Full details of the experiment are set forth in the accompanying table.

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
12-27-20	Dog No. 47.. Wt. 5.7 kg.	S. aureus....	No. 47	50	33	17	180	3.6
			Con.	50	37	13	199	3.9
		B. coli	No 47	50	45	5	140	2.8
			Con.	50	42	8	102	2.0
		B. bronchi-sept.	No. 47	50	23	27	38	0.7
			Con.	50	26	24	27	0.5

Date	Remarks	Bacteria	Animal	No. of cells counted	No. cells with bacteria	No. cells empty	Total no. of bact.	Phagocytic index
1 13 21	Dog No. 47. Wt 4.8 kg. 13 hours before death.	S. aureus	No. 47	50	36	14	162	3.2
			Con.	50	44	6	202	4.0
		B. coli	No. 47	50	46	4	146	2.9
			Con.	50	47	3	156	3.1
		B. bronchi-sept.	No. 47	50	48	2	110	2.2
			Con.	50	44	6	62	1.2
		B. typhosus	No. 47	50	11	39	12	0.2
			Con.	50	10	40	10	0.2
1 14 21	Dog No. 47. Wt 4.6 kg. Death	S. aureus	No. 47	50	41	9	163	3.2
			Con.	50	46	4	202	4.1
		B. coli	No. 47	50	47	3	163	3.2
			Con.	50	47	3	156	3.1
		B. bronchi-sept.	No. 47	50	46	4	112	2.2
			Con.	50	44	6	62	1.2
		B. typhosus	No. 47	50	5	45	6	0.1
			Con.	50	[illegible]	41	10	0.2

Autopsical Diagnosis. Marked emaciation, purulent exudate in nasal cavity and pharynx; extensive broncho-pneumonia. *Pasteurella canis* (P) cultivated from blood and from nasopharyngeal exudate.

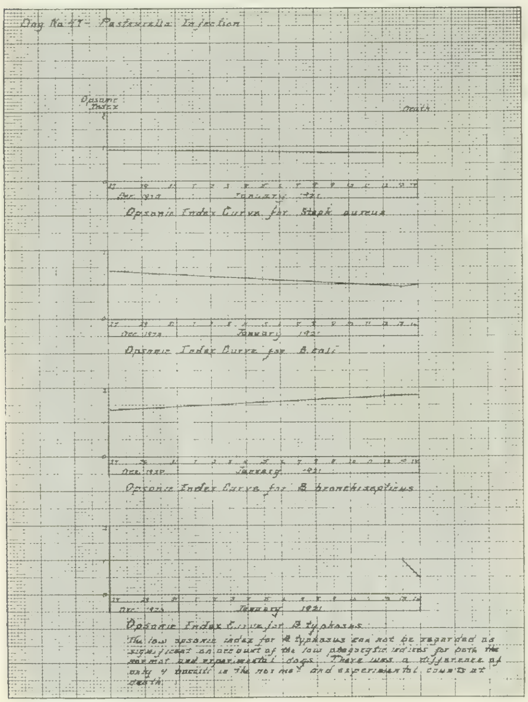

Fig. 7

It does not seem possible that any animals could attain a more defenseless condition than this dog presented during the last 36 hours of its life, and yet throughout this period there was no discoverable rupture in its phagocytic defense.

Discussion

Throughout this investigation it has been the custom in each enumeration to determine not only the total number of bacteria taken up by 50 neutrophile leucocytes, but also to record the number of these cells actually taking part in the engulfment. A survey of these records reveals in general a parallelism between the variations in the phagocytic indices and fluctuations in the percentage of phagocyting cells. Commonly a high phagocytic index is associated with a large percentage of phagocyting leucocytes, while a low index generally is obtained in preparations with few ingesting cells. However, this is not uniformly true and of the two methods for determining the degree of phagocytic activity it would appear from this research that the technique of counting the number of organisms ingested is the more delicate and reliable. It has frequently happened that the experimental animal revealed the same percentage of phagocyting cells as the control but yet exhibited the utmost variation in the total number of bacteria ingested.

There is always a question whether the opsonic results obtained by incubating samples of cells and serum with indifferent suspensions of bacteria can be accepted as indicating even approximately the conditions which prevail in the animal body. It is, of course, at once recognized that organisms grown on artificial media may differ fundamentally, in their reaction to living cells, from bacteria which develop within the body. Bordet[25] has shown, for example, that even when the most virulent organisms are injected into a susceptible animal, there is an initial phagocytosis. This he explains as a selective process. Supposing that any culture, no matter how virulent, contains numbers of feeble and weakly aggressive individuals and these the phagocytes at once select and devour. The remaining highly selected bacteria, then, in some cases, extend the infection without any phagocytic interference. Zinsser[26] likewise has pointed out that it is possible to obtain agglutination of "culture" pallida but quite impossible to do so when the spirochetes are taken directly from a lesion. A certain reasonableness therefore attaches itself to the objection that with phagocytic determinations *in vitro* one may be dealing largely with the enfeebled non-pathogenic fraction of the cultures examined and that the findings are worthless as an index of vital phagocytic defense. It cannot be denied, however, that opsonic determinations possess some significance and diagnostic value. It is possible that such observations in a specific case may be of uncertain value, but when extended over a large series, representing a wide variety in species and conditions, a uniform result gives ample justification for conservative deductions.

Perhaps the most striking feature of this investigation has been the unfailing effectiveness of phagocytic activity against the non-specific bacteria. In not one instance has there been a general decline in the opsonic indices for microorganisms not concerned in the primary infection. This has been uniformly true even in cases of extended infection, associated during the last days with the most destructive emaciation and with what appeared to be complete collapse of the animal's vital defensive mechanism.

It appears certain that the percentage of infected animals dying because of a collapse in the phagocytic defense is very

much smaller than is commonly supposed. Indeed, it is quite often as inaccurate and meaningless to say that an infected animal is overcome on account of a rupture in its phagocytic defense as it would be to contend that a man was run down by a train because of a break in his running technique, although at the time of the disaster he was exhibiting a speed beyond anything he had ever developed before in his lifetime.

Conclusions

The results obtained in this investigation would seem to warrant the following conclusions:

1. No decrease in phagocytic activity against any bacteria not concerned in the primary infection has been demonstrated even in the late stages of fatal infection. This would suggest that terminal infections do not arise primarily as the result of a rupture in the phagocytic defense.
2. A subnormal opsonic index for the specific infecting organism is not an invariable phenomenon in fatal infections.
3. Death in fatal infections does not always occur at the period of lowest opsonic activity.

In closing this paper I wish to thank Dr. W. G. MacCallum for his courtesy and assistance, and I desire to acknowledge a very especial debt of gratitude to Dr. S. Bayne-Jones for the substantial contributions he has made to the development of this problem.

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THE CHEMICAL DYNAMICS OF MUSCLE[1]

By F. Gowland Hopkins

In the previous lecture a few facts and some suggestions concerning oxidations in living tissues were discussed wholly from the standpoint of the chemical reactions involved. The biochemist must, however, recognize that the chemical reactions which it is his business to study occur in very peculiar apparatus. The structure of this apparatus is a factor—perhaps the most important factor—leading to that organization of chemical events which is the fundamental characteristic of any living unit. When the structure of a tissue is destroyed, oxidative processes may continue in the disintegrated material; but, as Warburg has remarked, they can then yield heat alone.

Specialization in the tissue-structure or apparatus may so influence the organization of chemical reactions—even of those which are common to all living tissues—that the final result of their progress, and the nature of the associated energy discharges, may be profoundly modified from case to case. The biochemist, while always concerned with the precise nature of the reactions themselves, must go further and study so far as he can their progress in the living tissue itself.

That this is not an impossible task is illustrated, I think, by chemical studies of striated muscle. Aided by thermodynamic studies they have already yielded knowledge, which, though very far indeed from affording a complete understanding of the relations which exist between the metabolism of muscle and its mechanical activities, is more than sufficient to show that experimental attacks upon such problems are justified. Viewed indeed from a purely chemical standpoint the dynamic events in this highly specialized tissue appear to be relatively simple, though the means which adjust them to a particular end are doubtless complex enough. Even in the latter connection, however, experimental studies are at the moment yielding interesting data of an unexpected kind, especially perhaps with regard to the functions of oxygen.

I ventured in my previous lecture to express disbelief in theories which refer the chemical manifestations of life to the properties of a hypothetical entity—the biogen or living molecule.

It was in connection with muscle when in 1867 Hermann put forth his *inogen hypothesis* that in an elementary form such theories arose. In my belief a fair consideration of the

[1] Lecture II of the Herter Series delivered before The Johns Hopkins University on April 13, 1921.

facts now known about the muscle is sufficient to prove them wrong.

You will of course remember that in Hermann's view the contraction of muscle follows upon the sudden, almost explosive, breakdown of a complex substance leading to the appearance of lactic acid and carbon dioxide. This unstable stuff is inogen, the source of energy. At first, influenced by his belief that the processes of Rigor were in essence similar to those of a Contraction, Hermann thought that the protein myosin, which separates in a gelatinous form during rigor, was part of the inogen. It is sufficient, however, to remember that the essence of Hermann's teaching was that the production of carbon dioxide in muscle depends not upon the direct and contemporary oxidation of relatively simple compounds, but upon the transference of oxygen, previously combined in a complex molecule, from less stable to more stable relations. Thus arose that conception of intramolecular oxygen which has given form to many biological speculations. Up to near the end of the last century the evidence seemed to justify it fully enough. Spallanzani, who seldom was in error, had shown even a century earlier that living tissues could long survive and continue to yield carbonic acid without any supply of oxygen except such as had been previously available. Spallanzani's statements were in later years fully confirmed by others, and Hermann made the evidence rather more definite in the case which especially interests us by showing that no trace of oxygen could be pumped out of a muscle which, nevertheless, could actively contract without an external oxygen supply, and at the same time produce carbon dioxide. Hermann's consequent views related in particular to the case of muscle, but as you well know, Pflüger later on (with other speculations as to the respective nature of dead and living protein) taught that all tissue respiration is based upon the activity of intramolecular oxygen.

Such views were almost unchallenged for 30 years, and since I am now to speak of work done in Cambridge, England, it is of interest to point out that, in a certain form at least, they satisfied the critical mind of him who founded this Cambridge school, as any who read the last edition of his famous text-book will discover. This edition had not long left the press, however, when one of the last of the brilliant group of workers directly inspired by Michael Foster began some work which was to change the whole outlook.

In 1898 H. M. Fletcher [2] published an account of an observation which, like many other observations of great importance, was in a sense of simple nature. The technique which made it reliable was not simple, however, and the value of the observation arose from its quantitative nature. Properly estimated it will in my opinion be found to have wholly removed the foundation of a belief which, as we have seen, had long dominated animal physiology. Excised surviving muscle gives out, it is true, a steady stream of carbon dioxide. It is clear, however, and it is an assumption, implicit if not explicit, in all that has been attributed to Hermann's inogen, that if this CO_2 arises from this hypothetical unstable source of energy, the amount should increase when the muscle passes from rest to activity. The breakdown of the inogen, which is *ex hypothesi* the source of energy, must surely be increased when the liberation of energy is increased. Therefore, since the inogen is also the source of the CO_2, a muscle, though wholly deprived of oxygen, must give out more CO_2 when it contracts than when it is resting. Fletcher showed that it did not.

The fact is fundamental. So long as a muscle remains in any sense normal, prolonged stimulation under anaërobic conditions fails to produce any effect upon its small but steady output of CO_2. Very different, as Fletcher further showed, is the case where a contemporary supply of oxygen is available. The output of CO_2 during rest is then greater and this output is at once increased when work is done. This difference between the chemical response to stimulation under anaërobic and aërobic conditions, respectively, is again fundamental. There is no evidence for the breakdown of a previously constructed inogen, but there is clear evidence that an increase in contemporary oxidations is associated with contraction.

But what of that relatively small but steady evolution of CO_2 which does after all occur anaërobically whether the muscle be active or at rest? It may be stated at once that it is not a product of metabolism at all. It is not physiological. When indeed we attempt later to picture the cycle of normal events, we may frankly forget it. It is merely carbon dioxide liberated from alkaline carbonates in the muscle as a result of the steadily increasing acidity which, as we are to see, is characteristic of the anaërobic conditions. To the best of my belief it was H. M. Vernon [3] who first suggested the possibility of such an origin: Hill afterwards referred to it, and later Fletcher [4] settled the matter by experiment.

Fletcher's early work provided further facts of significance. He fully confirmed the historical statements of Humboldt and later observations of Joteyko by showing that exposure to pure oxygen greatly delayed the onset of fatigue and of rigor mortis in excised muscle, but he went further and showed that a muscle which has lost the greater part of its irritability and is near to stiffening may be restored if exposed to pure oxygen.[5] From the facts which he made available Fletcher drew a conclusion which foreshadows an essential part of the story I hope to unfold. "It is suggested" he wrote in 1902, "that the hastening of rigor mortis and fatigue in a muscle from which oxygen is withheld is due to increased accumulation, under circumstances of deficient oxidation of the metabolic products within the muscle which are the potential precursors of carbonic acid"; while in the presence of oxygen "the dissociation processes alike of resting and active muscle, advancing slowly in the former, rapidly in the latter, result in the formation of free CO_2."

Since from the days of Berzelius lactic acid had been known as a constituent of muscle and many observers led by DuBois-Reymond had associated its production with activity and rigor,

[2] H. M. Fletcher: Jour. Physiol., 1898, XXIII, 10.

[3] Vernon: Science Progress.

[4] Fletcher and Brown: Jour. Physiol., 1914, XLVIII, 177.

[5] Fletcher: *Ib.*, 1902, XXVIII, 476.

Fletcher was of course alive to this probability that this acid is among those precursors of CO_2 which accumulates anaërobically, but he avoided at this time any dogmatic statement on the matter. The state of the literature as it then stood fully justified such caution.

Not long before the statement had been made by more than one observer that as a matter of fact there is no more lactic acid in a muscle in full rigor than in one perfectly fresh. Older views—which had never been supported by really quantitative work—were traversed and the significance of lactic acid in muscle had become entirely obscure.

A quantitative study was clearly necessary for further progress and in 1907 it was my privilege to join forces with Fletcher in an attack upon the problem.[6] Although the results of our somewhat laborious research have failed to affect the teaching of some text-books we have the satisfaction of knowing that they have been the acknowledged point of departure for recent important studies.

We found that the confusion in the literature as to the quantitative relations of lactic acid in muscle were wholly due to faulty technique in dealing with the tissue itself. When the muscle is disintegrated as a preliminary to extraction for analytical purposes, the existing equilibrium is entirely upset. Interacting factors are brought into abnormal relations and the processes of change are greatly accelerated. A fresh muscle had been supposed to contain as much acid as one in rigor simply because it had been produced in the former by the treatment which had preceded estimation. The biochemist had not sufficiently remembered the instability of his material. Fletcher and I, however, found it quite easy by means of a simple method, not only to avoid starting the changes which led to the formation of lactic acid, but to arrest them at any point during their progress and thus establish their time relations.

We were able to show that the accumulation of lactic acid in muscle occurs only in the conditions of anaërobiosis. With a proper oxygen supply it fails to accumulate at all, though this is not to say that it fails to be produced. Its formation anaërobically in a quiescent muscle shows no irregular relations: it is not, for instance, delayed until rigor appears, or is near. The accumulation starts from zero or from the minimal quantity present in fresh muscle and continues at a linear rate, equal amounts being produced in successive units of time. The reaction responsible for its appearance has a normal chemical temperature coefficient, at least between such temperatures as 10 to 20° C. At higher temperatures the effects of changes in the tissue structure become of influence and the process is accelerated so that at 40° C. there is a very rapid rise to that "acid maximum" which Kanke described long ago. At still higher temperatures and especially near 100° C. the process, like other biological processes, is arrested.

Stimulation increases the rate of production as should be expected. We have indeed every reason to believe, and I shall later give recent evidence to support the belief, that in muscle the chemical processes of activity differ quantitatively only, and in no way qualitatively, from those that proceed during quiescence. If a muscle be tetanized to complete fatigue Fletcher and I found that the lactic acid produced always stopped short of that maximum which is found in rigor mortis or produced rapidly at 40° C. In the fatigue of tetanus the amount is about half that of rigor (say 0.2 per cent as against 0.4).

Roughly to be classed as chemical stimuli are the effects of certain volatile organic substances, such as chloroform, toluol and others. When a muscle is exposed to the vapour of these a maximum yield of lactic acid is soon reached and a form of rigor is established, possibly because as solvents of lipoids these substances profoundly affect permeability relations within the muscle structure.

The next point established by Fletcher and myself is of fundamental importance. If a muscle which, by exposure to anaërobic conditions, has accumulated lactic acid, be placed in oxygen, the acid is removed. The occurrence of this removal under the influence of oxygen is significant in all that follows. It has been fully confirmed by the later work of Parnas and Meyerhof.

The experimental findings so far discussed have removed all basis from the theory of intramolecular oxygen, and have shown that an excised muscle is, at any rate, quite able to obtain free energy from its stores of potential energy without the aid of oxygen. On the other hand, while anaërobic existence, passive or active, inevitably involves an accumulation of lactic acid in the muscle, subsequent exposure to oxygen effects its removal. If, however, oxygen be available from the first, it must be remembered that the acid does not accumulate at all.

There are, therefore, at this stage of the enquiry perhaps two possibilities to consider. The muscle when forced to dispense with oxygen may be supposed to derive its energy from potential stores by chemical steps differing entirely from those which occur under physiological conditions. If so, lactic acid may be an abnormal product playing no part in normal events. On this view the anaërobic events are merely vicarious. But another possibility is that the normal cycle of events consists always of two phases separated in time: the first anaërobic during which lactic acid is formed, the second aërobic during which it is removed. Now the conception of a respiratory sequence in which anaërobic events precede and prepare the way for final oxidations has long been familiar to the minds of plant physiologists. Pfeffer, for instance, long ago spoke of the former as processes of intramolecular respiration, and looked upon them as necessary antecedents to subsequent oxidations. It is such a view that I propose to develop in connection with muscle, and I shall now proceed to give you further evidence in its favor.

But the facts won in the study of muscle indicate that the influence of oxygen has in the case of animal tissues more complicated relations than those usually postulated for the case of plant tissues: relations which seem to me to have extraordinary interest.

[6] Fletcher and Hopkins: Jour. Physiol., 1907, XXXV, 247.

At the close of our work upon lactic acid Fletcher and I were convinced that the influence of oxygen upon the activities of muscle is exerted after, rather than before or during, the actual act of contraction, and we were supported in this view by an observation previously made by Danilewsky [7] who found that heat is given out by a contracting muscle after the actual contraction is over: but the ordinary methods of chemical analysis seemed hardly capable of affording a final proof of this sequence. Clear evidence concerning the time relations of events could be best got from the study of a single contraction, a task impossible for the chemist.

At this stage, however, the Cambridge School of Physiology gained an unusual advantage by recruiting, in the person of A. V. Hill, one who is at once an accomplished mathematician and a skilful experimentalist. Hill's qualifications have enabled him to advance very greatly the thermodynamics of muscle and to supply highly essential links in the chain of evidence which has led to the standpoint I am to advocate.

His studies have been made partly on the lines of micro-calorimetry, but also, and more particularly, by the use of thermoelectric methods which he has greatly refined. A fundamental observation made early in Hill's researches puts upon a firm experimental basis the conclusion of Danilewsky already quoted, namely that the heat given out by an active muscle long outlasts the contraction itself.[8] But Hill's further time analysis of the thermal phenomena is so important to my thesis that it should be very fully grasped at least in its essentials. The essentials are these. Directly associated with a contraction is a rapid evolution of heat, which occurs both under anaërobic and aërobic conditions, and is, moreover, alike in its time relations and other characters, wholly unaffected by the presence or absence of oxygen.

Oxygen, however, has a noteworthy effect upon the sequel. In its absence the heat evolution just mentioned is quickly over, and is the only thermic change associated with the act of contraction. In its presence there is a further evolution of heat subsequent to, and long outlasting, the act. These facts are clearly of great significance. The process which comes first must be anaërobic: that which comes second can scarcely fail to involve an oxidation. We have here a proof of the suspected sequence of events, and data which strongly suggest that at the moment of contraction, even under conditions made normal by the available supply of oxygen, something is produced anaërobically upon which oxygen subsequently acts. The chemical facts already discussed leave but little doubt that this substance is lactic acid, and evidence will be immediately given to show that the formation of the acid is as a matter of fact intimately associated with the process which gives rise to the early anaërobic heat production. Later on we shall see that there are abundant reasons for associating the second oxidative heat production with the occurrence of its removal.

Already we may speak, at least provisionally, of two phases in the muscular act, separated in time—an anaërobic phase of activity, or *fatigue phase,* and an aërobic phase, or *recovery phase.* It is convenient to make this distinction now. Further justification for it is inherent in all that follows.

The recovery phase must not be confused with the relaxation of the muscle, which, like the contraction proper, is primarily independent of oxygen and is included in the first phase. I shall deal first with this phase of activity.

Early in his studies Hill demonstrated a fact which yields an important point in the evidence. Between the mechanical tension which appears in the muscle and the production of heat, there is always a direct proportionality. Alike in the steady anaërobic metabolism of the quiescent muscle, or during stimulation, or in anaërobic various forms of rigor however rapidly induced, the appearance of a given quantity of acid is associated with the evolution of a proportional quantity of heat. A specially accurate quantitative proof of this was given by the work of R. A. Peters [9] at Cambridge. Using admirable technique he showed that while the indirect stimulation of muscle to fatigue involved the evolution of 0.9 cal. per gram of tissue, the subsequent induction in the same muscles of rigor by chloroform added 0.89 cal. per gram. When rigor was directly induced in fresh muscle the heat production amounted almost exactly to the sum of the above, and double the heat of fatigue, namely 1.70 cals. Now Fletcher and I had shown, as already mentioned, that in rigor the average production of lactic acid is about double the production in fatigue, and this Peters confirmed. Each gram of acid when it appears in muscle is accompanied by the appearance of about 450 calories, and the constancy of this ratio under such different conditions shows that acid and heat owe their origin to one and the same process. Equally important in the development of the views I am putting before you is the indication supplied by such results that the events which give rise to lactic acid formation during normal activity are in no sense of a different order from those which are responsible for the appearance of the acid during the anaërobic metabolism of rest, or in rigor however produced or accelerated.

These results have been in all essentials amply confirmed by the very recent work of Meyerhof,[10] to which in other connections I shall have to make frequent reference.

His experiments have an advantage over the earlier ones in that the estimations of heat and lactic acid were made upon the same set of muscles, and the comparison was direct. At the same time it must be remembered that only micro-methods could be used for such a purpose. Meyerhof found, as the result of change in conditions such as the temperature at which the muscles are stimulated, certain variations in the heat evolved per unit of lactic acid produced, but proved that the relation was essentially of the same order as that assumed by Hill and Peters. With regard to the effect of temperature Hill has very recently shown that in the response to a single

[7] Danilewsky, Pflügers Arch., 1889, XLV, 353.

[8] A. V. Hill: Jour. Physiol., 1910, XL, 389; with series of subsequent papers in the same Journal.

[9] Peters: Jour. Physiol., 1913, XLVII, 243.

[10] Otto Meyerhof: Pflügers Arch., 1919, CLXXV, 20 and 88; *Ib.*, 1920, CLXXXII, 232 and 284.

shock the relation is the same at all temperatures. In prolonged stimulation it varies. For average conditions we shall be close to the mark if we take, with Meyerhof, 400 small calories as the heat production which is normally associated with the formation of 1 gm. of lactic acid.

Lactic acid is related in a similar quantitative way with mechanical changes in the muscle. To get clear on this point we must realize certain properties of the muscle as a machine which have been brought fully to light by the investigations of Hill. Earlier experiments had shown that the actual work performed by a contracting muscle bears very variable and apparently accidental relations to the heat evolved. The case is different, however, if we deal with the primary mechanical event which follows immediately upon stimulation and precedes any performance of work. This is a sudden increase of tension in the fibres which can be accurately measured. It means that the elasticity of the fibres has suddenly changed, and its occurrence implies a sudden conversion of chemical potential energy into mechanical potential energy. The subsequent developments in the muscle depend upon the circumstances in which it is placed. If it be free to contract, then part of the energy of the new tensile stress will appear as work and part, of course, as heat. If the muscle after stimulation cannot contract, the whole appears as heat, Hill having established the important fact that the heat evolution is then always proportional to the amount of tension previously established in the muscle. Much, I may say here, of what is quantitative in our present knowledge is due to Hill's recognition of the importance of studying the dynamics of the isometric contraction. Such studies have yielded proof of the relation just mentioned.

Since the mechanical potential energy established in the isometric muscle by stimulation degrades into a proportionate amount of heat, and this heat has been shown to be proportionate under all circumstances to the lactic acid produced, it follows that the original mechanical potential is proportionate to acid production. This Meyerhof has directly shown by estimating the integrative effects of a series of isometric contractions carried on to the point of fatigue. He found that the total mechanical effect is in general proportionate to the total acid formed, the absolute value of each varying with such factors as the nutrition condition of the animals used.

The relations just discussed compel the belief that acid production, the development of tension, and the heat which ultimately leaves the muscle, are all essentially due to one event and that a chemical change following immediately upon excitation. Here clearly we reach a standpoint based upon quantitative data of the utmost importance for the understanding of the dynamics of muscle.

Our next desire will naturally be to discover the nature of the primary chemical reaction concerned. We need then first to know what is the precursor of the lactic acid. All the probabilities would point to carbohydrate as the ultimate, if not the immediate, source of the acid, but the experimental evidence supposed to bear on the matter seemed for a long time hopelessly confused. It is not necessary to enter into the history of controversy on the point. Much of it has been due to the fact that the data upon which arguments have been based were yielded by unsatisfactory methods and so were contradictory and without quantitative value. Recent work and especially that of Parnas and Meyerhof shows at least that whenever lactic acid appears in muscle an equivalent quantity of carbohydrate disappears. These observers used micro-methods for their estimations, though their results were carefully controlled. In my laboratory we have recently repeated the observations using similar methods with larger quantities of material. We have been impressed by the exactness with which the amount of lactic acid formed under any circumstances corresponds with the amount of carbohydrate simultaneously disappearing.

Seeking detail as to the exact nature of the precursor we may pass at once to the results of the patient investigation of the Frankfort School. Doubtless having in view the classical discovery of Harden and Young, who showed that in the chemical breakdown of sugar by yeast juice the synthetic formation of a phosphoric ester of hexose is an invariable event, and also being aware of certain data in the literature which suggested that during the activity of muscle inorganic phosphates may be increased at the expense of phosphates organically bound, Embden and his colleagues, Laquer and others, set themselves to look for hexose phosphate in muscle.

The final outcome of a patient and laborious investigation was the proof that this hexose phosphate does undoubtedly exist in muscle and shares in the processes of change.[11] Moreover, muscle-extracts and other tissue-extracts, while quite incapable of converting free sugar into lactic acid, break down the hexose phosphate in such a way as to yield both lactic and phosphoric acids. The view may be taken, and if true, it is one of great interest, that the association with phosphoric acid in some way makes easier that intramolecular shift which is involved in the production of lactic acid from dextrose. We can hardly doubt, therefore, that between the glycogen and the lactic acid of muscular metabolism this combination of sugar with phosphoric acid is an intermediary. It is probably the immediate precursor of the latter and is in any case a precursor at some stage in the processes of production. Its properties justify the title "lactacidogen" which Embden has applied to it.

In their more general aspects the occurrences of the active phase of a muscle act may be pictured as follows: As the result of stimulation, sugar originally derived from the glycogen stores but situated in some position of advantage in the muscle structure and almost certainly combined there with phosphoric acid suffers a sudden breakdown yielding lactic acid. As a result of this chemical event the muscle elements suffer a marked change in respect of their elastic properties and this leads to a development of mechanical tension within them proportionate in amount to the acid produced. This tension may then be converted into work, but in such conditions as those which exist when a muscle contracts isometri-

[11] Embden and Laquer: Zeitsch. f. physiol. Chem., 1916, XCVIII, 181; 1921, CXIII, 1.

cally it is wholly degraded into heat. This heat, though by no means to be thought of as identical with the heat of the initial chemical reaction, is always proportionate to the extent of that reaction, and so also to the lactic acid produced. So long as the events of the next phase—the recovery phase—are for any reason in abeyance, the acid accumulates in the muscle. But these events, in which the influence of oxygen is dominant, must be now considered.

The recovery period is most easily pictured when conditions sharply demarcate it in time, as when a muscle properly supplied with oxygen is engaged in a series of single contractions. It then intervenes between relaxation and the following contraction. But the evidence shows that the chemical processes involved in it, so long as oxygen is fully available, continuously play their part in the chemical equilibrium of muscle whether quiescent or active.

The facts already discussed have shown that the main event during recovery is the disappearance of lactic acid. But the precise mechanism of its removal is a matter vital to our understanding of the dynamics of muscle. If, for instance, we endeavour to take the simplest view of the situation and in analogy with what has been taught concerning plant respiration conceive that the sequence of events is merely that of acid production during activity, followed by complete combustion of the acid, during recovery, we meet at once an inherent difficulty which has prevented many in the past from recognizing in lactic acid a normal product of activity.

The demands of a working mechanism such as muscle are somewhat different from those of more quiescent plant tissues. It calls not only for a supply of available free energy but for the delivery of this at the right moment.

Now in the breakdown of carbohydrate to yield lactic acid a very small fraction, perhaps one-fortieth, of the chemical potential of the former is made available as free energy. The rest would be set free during the subsequent combustion, but as the act of contraction has *ex hypothesi* preceded this, the natural conclusion would be that the mechanical activity receives no support from oxidations, which then would provide heat alone.

Under such circumstances the consumption of material necessary to yield a given amount of work would be out of all proportion greater than what is known to occur in the normal intact animal.

If, then, the splitting of carbohydrate into lactic acid as a source of energy for the contraction be no abnormal process, vastly uneconomical, to which the muscle is merely driven by deprivation of oxygen; if the sequence of events we are picturing be real, then in some way, energy must somehow be made available for activity during what we have called the recovery phase. Recent research has made it clear that there is a real post-contractile return of material and energy to the system of the muscle. The facts are of great interest and would seem to bear not alone upon the dynamics of muscle but no less perhaps upon the nature of the relations between oxygen and living processes in general.

During our work upon lactic acid Fletcher and I obtained an experimental result which seemed to us significant. We submitted muscles to processes of alternating fatigue and recovery repeated many times. As during each period of recovery lactic acid left the muscles it is clear that the treatment referred to if it involved complete oxidation must ultimately have made a heavy drain upon the sources of the acid. Organs so treated might be expected to show sign of consequent deficiency in these. Yet when they were warmed to 40° C., so as to obtain from them Ranke's "heat maximum" it was found that they yielded just as much lactic acid as perfectly fresh unfatigued muscles. Though we tried not to dogmatise upon the matter, we thought that here was some justification for believing that the acid was, during the recovery periods, not burnt away, but perhaps rebuilt, not, of course, with the oxygen into an inogen, but into whatever substance had first yielded the acid anaërobically. It was afterwards shown, however, by Laquer that a simpler explanation for the facts is available. The production of lactic acid of muscle is a self-regulated reaction stopping short when a certain maximum is reached. If, therefore, the original supply of precursor is relatively large, we need not expect that its partial exhaustion will appreciably reduce the final maximum. There are certain considerations to suggest that the experimental result described is not entirely to be explained in this way; but I need not dwell upon them because there is now other and more direct evidence to show that the processes of recovery reverse the processes of fatigue.

Quite early in his researches Hill measured the heat given out in the period which follows a single muscular contraction, and found this so-called *heat of recovery* to be quantitatively of the same order as the *heat of contraction*. To this result he applied the following argument: The lactic acid produced in contraction disappears during recovery and since the heat associated with the formation is known to be about 400 cals. per gram, while the heat of recovery is about the same, only some 400 cals. can be evolved during the removal of 1 gm. But the total heat of combustion of 1 gram of lactic acid is something like 3700 cals. It is impossible, therefore, that more than a fraction of the acid which is removed during recovery can be actually burnt. Most of it must be removed in some other way; as Hill thought, by reconstruction into a substance of higher potential energy.

But before the argument can be completed, more precise and direct information is wanted as to how much material is actually oxidised in the recovery phase. This is not necessarily measured by the heat given out. It can be determined, however, by measuring the amount of oxygen consumed, especially if at the same time we get to know the respiratory quotient. Such a determination was first made by Parnas[12] in the course of work begun at Cambridge. Parnas found first of all that the oxygen consumption of an excised muscle, resting, but previously fatigued and therefore loaded with lactic acid, was definitely greater than that of a fresh resting muscle containing at most a minute quantity of acid. His results seemed

[12] Parnas: Centrlb. f. Physiol., 1915, XXX, 1.

to show that this excess in the oxygen consumed corresponded with some exactness to the combustion requirements of the amount of lactic acid known to have disappeared from the fatigued muscle by the end of the experiment. But he found also, and here he demonstrated a point of great importance, that the heat actually evolved was considerably less than what this combustion would produce; the former being indeed only half the latter. Parnas drew from his results the conclusion that while the lactic acid was not resynthetised, but wholly burnt, the energy of its combustion was not all lost to the muscle. On the contrary, a moiety of this energy is by some unknown means employed in restoring potential to the contractile system.

You will not deny that this return of energy to the physical mechanism under the influence of oxygen is a significant event. Its occurrence has been confirmed by the later work of Meyerhof.

In a series of papers which have appeared during the last year this author, who had previously identified himself with productive studies of general tissue-respiration, has made important contributions to the subject of this lecture. Much of his work constitutes a confirmation of that of Fletcher and myself, as also that of Hill and Peters; though, especially in connection with the effect of conditions on lactic acid formation, he has added precision to details. But he has also added facts, and especially one fact of great importance.

Meyerhof repeated Parnas's experiments on the oxygen consumption of the recovery period, estimating directly in the the lactic acid which disappears. The conclusion that part of the energy arising from the oxidation is retained in the muscle was confirmed, but Meyerhof's results differed quantitatively from those of Parnas, and the difference modifies the interpretation of the facts. Meyerhof agrees with Hill in finding that the observed heat of recovery is of the same order of magnitude as the heat of contraction, though his results suggest that the former is somewhat the greater, in the ratio, say, of five to four. He finds with Parnas that this value is considerably less than what would correspond with the lactic acid (or carbohydrate) burnt, but the figures he obtained for oxygen consumption indicate that not the whole but no more than one-third to one-fourth of the lactic acid is actually burnt; the remainder disappears without oxidation. This was Hill's view. But Meyerhof has gone beyond previous observers by showing what this disappearance means. The balance of the acid he finds is actually reconverted into glycogen!

E. J. Lesser,[13] several years ago, showed that the glycogen of intact frogs was greatly reduced as the result of a comparatively brief deprivation of oxygen, and that when the animals were returned to normal respiratory conditions, without receiving food, the total glycogen returned in part to its original value. This might possibly indicate a resynthesis from lactic acid formed during the anoxybiosis. But the process in the whole animal might have been quite indirect, and involve other organs than the muscles. Meyerhof on the other hand, by simultaneous estimations of lactic acid and glycogen in excised muscles, before and after their recovery in oxygen from previous fatigue, has shown directly that during the process the one is actually reconverted into the other.

[13] Zeitschr. f. Biol., LI, 287.

Meyerhof's results have been fully confirmed in my laboratory by the conjoint work of Miss D. Foster and Miss Moyle.

Energy, it would seem, is returned to the muscle along two lines; one involving a restoration of chemical potential, and the other the restoration of potential to a physical system. Each event has its own importance in the return to the *status quo ante*.

From the chemical standpoint the equilibrium changes involved during activity and recovery resolve themselves ultimately into the simple reversible equation:

$$\text{Glycogen} \leftrightarrows \text{Lactic acid.}$$

So long as anaërobic conditions prevail the production of lactic acid is an irreversible process; under the influence of oxygen it becomes reversible, though at the cost of the oxidation of a part (say one-fourth) of the reacting materials.

We may provisionally put the relations just discussed into quantitative form with the data of Hill and Meyerhof as a basis. Calculated as for one gram of muscle the average of Meyerhof's results gives for the heat of contraction 0.75 cals.; for that of recovery 1.0 cal.; and for the heat of the lactic acid (or carbohydrate) actually burnt during recovery 1.75 cals. If we calculate in round figures the calories corresponding to 1 gram of lactic acid either produced or removed we get the data in a convenient form:

Heat of anaërobic contraction	Heat of recovery in oxygen	Total heat of activity in oxygen	Heat of combustion of materials burnt
400 cals.	500 cals.	900 cals.	900 cals.

This is a balance sheet which any auditor would pass as satisfactory. The heat lost as the result of an exothermic change during the fatigue phase (400 cals.) is balanced by the absorption of an equal amount of heat due to an endothermic change during the recovery phase. On the other hand, the heat of combustion of materials actually shown to have been oxidised exactly provides the total loss of energy (900 cals.) during the whole cycle of change (400 + 500 cals.). As a result of the recovery processes, therefore, the muscular system is restored to the condition which existed before activity, except for the fact that, in the disappearance of part of its glycogen store, it has lost chemical potential energy equivalent to the energy it has expended.

There is temptation at this stage to make some attempt towards bringing the chemical facts and the related thermal data which have been discussed into some definite picture of the whole mechanism of contraction. To do so would involve entering a field which is still largely speculative, and any such attempt to be worth while would call for much time. It will be well, therefore, if I continue to confine my remarks within the limits indicated by the title of the lecture and deal with chemical aspects of the subject alone.

I would like, however, just to make the claim that, properly estimated, the evidence available should be held sufficient to

make it probable that at some point in the cycle of muscular activity the appearance of lactic acid directly influences the mechanical events by inducing a sudden change in the hydrogen-ion concentration at contractile surfaces. If so, there is much interest in the relation of the muscular machinery to the fuel it consumes.

We possess suggestive if not wholly conclusive evidence which indicates that lactic acid is an obligate intermediary in the breakdown of carbohydrate in all animal tissues.

We may therefore suppose that in the evolution of the structure of striated muscle advantage was taken of the existence of this acidic metabolite. Structure and metabolism became mutually adjusted to produce a remarkable and highly efficient machine, in which, at one stage in a cycle, part of the fuel which is to be ultimately burnt becomes temporarily part of the machinery—at this stage it is concerned with the liberation rather than with the supply of energy. It would seem further that to exert its special influence as an acid the metabolite must at a given moment be produced in much larger amount than what is required for immediate oxidation: the balance is therefore returned to the store.

Intimately connected with these peculiar relations between fuel and machinery are the special time relations of the oxidative process to which full attention has been given in the lecture. Oxidations do not immediately and directly supply the energy of contraction. They rather restore potential. They, as it were, wind up the clock: or if you prefer the analogy, oxidations yield energy as does a dynamo when it changes an accumulator. The store in the accumulator may be what actually runs the machine.

In spite of the simplicity in events suggested by the direct relation between carbohydrate and lactic acid in muscle metabolism there is, even from the standpoint of chemistry alone, much yet to be learnt concerning details. If it does not deal with its carbohydrate quite differently from less specialized tissues, we must look somewhere amid the dynamical events, which in muscle connect lactic acid with glycogen or hexose phosphate, for the influence of that catalyst which controls equilibrium between lactic acid and methyl-glyoxal. The discovery of this catalyst by Dakin and its study by Dakin and Dudley constitute one of the most significant contributions ever made to the chemistry of the tissues. Until we know where in the sequence of events the activity of this cell catalyst finds a place, we must in my opinion be content with an incomplete picture of chemical dynamics in the cell.

Mention of glyoxalase leads me to consider very briefly another fundamental matter upon which the study of excised muscle has a bearing. Dakin and Dudley showed, as you will remember, that nearly all functionally active tissues contain this enzyme, so that extracts made from them rapidly convert methyl-glyoxal into lactic acid. The pancreas stands out as an exception. Not only is it probably free from glyoxalase but it contains a factor which inhibits the action of that enzyme. Pancreatic extracts can prevent the formation of lactic acid from methyl-glyoxal by other tissues. Now Winfield and I have shown that pancreatic extracts diminish the velocity of lactic acid formation in chopped muscle, the source of the latter being, as Meyerhof has proved, undoubtedly the carbohydrate of the tissue. The facts suggest that methyl-glyoxal and lactic acid are both intermediate products in the breakdown of carbohydrate and that the pancreas may control the velocity of this breakdown at a stage between the appearance of methyl-glyoxal and its conversion into the acid.

It is not yet clear how we are to relate these possibilities to the occurrence of glycosuria; but it would be wrong, I think, for those who are concerned with diabetes to ignore them.

We shall not understand the whole of the fascinating chemical system which has occupied our attention if we think only of its energy exchanges and the material events which support them.

All the dynamic phenomena we have considered, though they may be displayed to perfection for long periods by muscles excised from the body, are of course displayed only while a certain equilibrium is maintained among a complicated set of factors; the equilibrium which is associated with, and necessary for, the property of irritability.

So long as this is maintained, or, if lost, is capable of restoration, so long are we justified in attributing life to the tissue. A moment must come when the necessary equilibrium is no longer maintained, and this moment is usually—though, as we are to see, not always—associated with, and marked by, a happening which seems to have a more or less critical character—the rigor of death. This may arise as the final result of oxygen lack alone; but we have learnt to make no sharp distinction between the onset of rigor mortis and that gradual progress of fatigue which in active muscle may lead up to it: a progress which is accelerated by deprivation of oxygen and delayed by a proper supply of that essential factor.

It is the accumulation of products of change and not the exhaustion of supplies of oxidisable material which leads to fatigue and ultimately to death in rigor. Fletcher's early work and the work we did together, as well as such observations as those of Joteyko, left little doubt that the prime if not the sole cause of fatigue, and, no less, of death in rigor, is the accumulation of lactic acid. Both phenomena are due to the effect of this upon the colloid machinery of the muscle. Fatigue increases as the accumulation increases and the critical moment of rigor mortis (in so far as it is critical) corresponds with the attainment of a certain concentration of acid.

These are the common and obvious events. When they occur they overshadow other failures in equilibrium which may nevertheless be as fatal to what we call the life of the tissue. An excised amphibian muscle, when quiescent in an atmosphere of oxygen, does not accumulate lactic acid, and never displays rigor mortis at all. Yet though it lives surprisingly long, it is certainly not immortal. It ultimately ceases to be irritable and we must then speak of its death; but it is death without rigor. What now is the cause of death? Miss D. L. Foster and Miss D. M. Moyle have carried out in my laboratory some experiments which bear upon the answer to this question and the results seem to be of great interest.

If frog's limb muscles are kept in oxygen at 0° C. they remain irritable for periods which vary with the season from about a fortnight to three weeks. The irritability declines relatively fast immediately after the first few days, then remains constant for a period, and later declines more rapidly to zero. But the muscles when, finally, they fail entirely to respond to the strongest electrical stimulus show no signs of rigor mortis. In their flaccidity and in their general appearance they resemble perfectly fresh muscles. Corresponding with this they are found to contain only that original "resting minimum" of lactic acid which must have been present when they were originally removed from the frog. In parenthesis it may be stated that this failure to accumulate lactic acid is not due to the complete inhibition by the low temperature of the chemical processes concerned. In nitrogen the muscles at 0° C. accumulate lactic acid at a steady rate.

What, however, is noteworthy in the condition of muscles which have lost excitability during long exposure in oxygen at 0° C. is the fact that their chemical mechanisms remain intact. Placed in air at 40° C. they rapidly come to exhibit the normal maximum of lactic acid, and they show no less the usual acid production which results from injury, or, say, the action of chloroform vapor. Although they transmit no change of electric potential, they show, in further proof of their chemical integrity, a demarcation current when injured. Judged by chemical criteria they are alive, while tested by electric stimulation they appear to be dead. There is, as it were, a dislocation between stimulus and energy discharge. A narcotized muscle may be said to show a similar dislocation; but the underlying conditions are in this case different; for narcosis encourages the accumulation of lactic acid. What then is the state of affairs in the inexcitable muscles we have been considering? One hypothesis suggests itself. Nernst's theory postulates the free movement of ions towards a critical concentration at a surface as an essential happening in the excitation of a tissue. Now in systems containing both colloids and electrolytes in solution there is a tendency with progress of time for the associations between them to assume increased stability. This may occur in muscles which remain for long periods at 0° C., thus ultimately the concentration of ions which are free to move may become too small to form the necessary assemblage at a surface. Some confirmation of such a view is to be found in the fact that, though the muscles in question have suffered no loss of material save a small fraction of their glycogen, the osmotic pressure in their fibres is much less than that which they exhibit when first removed from the animal.

NOTES ON NEW BOOKS

A Diabetic Manual for the Use of Doctor and Patient. By Elliott P. Joslin. 2d ed. (*Philadelphia and New York, Lea and Febiger, 1919.*)

The second edition of this little book is an indication of its continued usefulness. Here in a few pages, the writer in his forceful epigrammatic way has summarized most that is of practical value in the management of the diabetic. Throughout there is a background of personal experience which lends weight to the dicta expressed in the text. A study of this book will keep the physician, who does not specialize in diabetes, on the right track when called on to handle the occasional case, and the patient himself can glean from it sound counsel without becoming over-educated. A. L. B.

Diabetes, a Handbook for Physicians and Their Patients. By Philip Horowitz. Cloth, $2.00. (*New York, Paul B. Hoeber,* 1920.)

This little book contains a useful summary of the author's methods of handling diabetics. The subject is well presented, but adds little to previous works of similar character. A. L. B.

The Oxford Medicine. By Various Authors. Edited by H. A. Christian and J. Mackenzie. (*New York, Oxford University Press.*)

This volume keeps up the general excellent standard of its predecessors. The subjects, including diseases of the gastro-intestinal tract, liver, pancreas, and peritoneum are adequately treated. The articles on nephritis are of outstanding value. Here for the first time in a text-book do we find a modern consideration of the subject based on the facts brought out by recent chemical and clinical studies. The writer has wisely taken up the subject from the clinical standpoint, and avoided the confusion of the combined etiological, pathological, clinical classification which we usually find. A. L. B.

Heart Affections. Their Recognition and Treatment. By S. C. Smith. Cloth, $5.00. (*F. A. Davis Company, Philadelphia, 1920.*)

In this little compendium the writer has adopted a rather new method of exposition. Without going at great length into experimental fundamentals, he brings the more modern methods of cardiac study en rapport with pure clinical observation. While hardly complete enough to serve as a treatise for the beginner, the book seems to us most valuable to the practitioner who is accustomed to the point of view of fifteen years ago and desires to add to his armamentarium the newer physical methods of study. The material is clearly presented and while the pure cardiologist might take exception to small points, perhaps more on account of their incompleteness and lack of qualification than for other reasons, the book seems to us to have accomplished well the author's purpose. **A. L. B.**

The Treatment of Acute Infectious Diseases. By F. S. Meara. Second Edition. (*MacMillan Company, New York, 1921.*)

Text-books on treatment are in the main unsatisfactory; either they are brief, inadequate outlines, or they catalogue at length a variety of possible measures without due criticism. It is a delight therefore to read Meara's admirable treatise, and to find there detailed practical directions as to just what to do, based on sound scientific principles, but tempered by the author's extensive experience. The introductory sections on fever and on diet in infectious disease strike fundamental keynotes which recur in the succeeding sections. As to the actual substance of the book the reviewer finds himself in complete agreement with the author's ideas and methods. The detailed method of exposition, especially with reference to local measures and modes of drug administration, is highly satisfactory. A good deal is said about vaccine therapy, but it is only fair to say that the writer makes no undue claims. Finally, one of the most pleasing features of the book is its readable style which allows one to peruse its successive pages without weariness. A. L. B

The Principles of Immunology. By H. T. Karsner and E. E. Ecker. Cloth $5.00. (*J. B. Lippincott Company, Philadelphia and London, 1921.*)

This book represents an up-to-date summary of its subject. As stated by the authors, previous works have been freely drawn on and many sections are reminiscent of other compendiums. The work seems to be of distinct value, combining as it does theory with practical conclusions and confining itself to that which has been pretty clearly established as fact. A. L. B.

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EXPERIMENTAL INOCULATION OF HUMAN THROATS WITH VIRULENT DIPHTHERIA BACILLI

By C. G. Guthrie, B. C. Marshall and W. L. Moss

(From the Division of Clinical Pathology of the Medical Clinic, The Johns Hopkins University and Hospital)

In previous reports * we have called attention to certain points concerning healthy carriers of diphtheria bacilli.

1. Such carriers are relatively abundant in the community, as may be readily demonstrated by taking throat cultures from a large series of individuals, particularly during the winter months. The number of carriers revealed by a single examination may be approximately doubled by a second examination of the same series of persons, and a third examination still further increases the number found.

2. The great majority (80 to 90 per cent) of these healthy carriers harbor diphtheria bacilli which are non-virulent. These non-virulent diphtheria bacilli are to be distinguished from the virulent ones only by their inability to produce toxin. So far as we know, this differentiation may be accomplished only by the inoculation of susceptible animals.

3. Among all of the healthy carriers of diphtheria bacilli, virulent or non-virulent, studied by us, none developed clinical diphtheria during the period of our observation, although this was in many cases extended over several months and even years.

4. A painstaking investigation failed to reveal a single case of clinical diphtheria developing among any of the associates of the healthy carriers under our observation.

5. By inoculating the throats of a series of five human volunteers with non-virulent diphtheria bacilli obtained from the throat of a healthy carrier it was shown that:

(a) The carrier state was readily produced in all, lasting from 11 weeks to 15 months or more.

(b) The development of the carrier state was not prevented by the previous injection of antitoxin in certain members of the group.

(c) Confirmation of the guinea-pig test for the avirulence of this organism was obtained by these experiments, in that this organism which was shown by repeated tests to be avirulent for guinea-pigs failed to produce (a) clinical diphtheria; (b) any subjective symptoms; (c) any objective changes in

* Moss, W. L., Guthrie, C. G., and Gelien, J.: Diphtheria Bacillus Carriers, Trans. XV Internat. Cong. on Hyg. and Dem., 1912, IV, 156.

Guthrie, C. G., Gelien, J., and Moss, W. L.: Diphtheria Bacillus Carriers, Second Communication. Johns Hopkins Hosp. Bull., 1920, XXXI, 388.

Moss, W. L., Guthrie, C. G., and Marshall, B. C.: Experimental Inoculation of Human Throats with Avirulent Diphtheria Bacilli. Johns Hopkins Hosp. Bull., 1921, XXXII, 37.

Moss, W. L., Guthrie, C. G., and Gelien, J.: Diphtheria Bacillus Carriers. A Report of Conditions Found in an Orphan Asylum. Johns Hopkins Hosp. Bull., 1921, XXXII, 109.

the appearance of the throat when inoculated and colonized in human throats.

(d) No cases of clinical diphtheria developed among the associates of these artificially produced "healthy carriers" of avirulent diphtheria bacilli.

(e) When isolated in pure culture after prolonged sojourn in the throats of these carriers, the bacilli were found unaltered in morphology, in staining or cultural characteristics, and showed no tendency to become virulent, either in the carriers who had received preliminary injections of antitoxin or in those who had not.

6. The results of the foregoing observations on individuals in the carrier state, whether spontaneously or experimentally produced, warranted the general conclusions that non-virulent diphtheria bacilli are incapable of producing clinical diphtheria and that carriers of such organisms are not a factor in the spread of the disease.

These conclusions still leave for consideration, however, the healthy carriers of virulent diphtheria bacilli regarding whom certain questions naturally arise. With the exception of diphtheria bacilli isolated from actual cases of clinical diphtheria we have in the past relied on the guinea-pig test for determining the virulence or avirulence of a given strain. The question has often been raised as to whether the demonstration of virulence for the guinea-pig may be taken as proof of virulence for man. While the validity of the guinea-pig test as indicating virulence for man is generally accepted and there is much evidence in its favor, the conclusive proof, so far as we are aware, had never been brought and certain facts seemed to leave the question an open one. These facts may be indicated by stating the questions which arose concerning healthy carriers of virulent diphtheria bacilli. The first question concerns the reason why such carriers do not themselves develop clinical diphtheria. Is it because they are protected against the effect of diphtheria toxin by means of an immunity either naturally present prior to infestation or gradually acquired as the result of the presence of virulent bacilli in the throat? Or is it because the virulent organism from the throat of a healthy carrier, though virulent for the guinea-pig, differs in some way from the virulent organism from a patient with diphtheria and so is incapable of producing the disease in human beings? In other words, is the guinea-pig test misleading in this respect? The second question concerns the reason why no discoverable case of clinical diphtheria occurred among the associates of the healthy carriers of virulent diphtheria bacilli. This question again brings up the validity of the guinea-pig test for virulence. Do such carriers fail to transmit the disease to others because the organisms which they harbor, though virulent for the guinea-pig, are incapable of producing diphtheria in man? It was in an endeavor to find the answer to these and certain other questions that the present work was undertaken. It is largely a repetition of the series of experiments carried out in the previous year (1913), in the course of which we inoculated the throats of human beings with non-virulent diphtheria bacilli. The only essential difference lies in the strain of organism used for our inoculations, which, in the work to be presented here, was a virulent diphtheria bacillus obtained from the throat of a healthy carrier. Since it parallels the previous work so closely, the form of the earlier report is also followed here; in many places the identical wording is retained.

Object

1. To determine whether the introduction of virulent diphtheria bacilli (as determined by guinea-pig inoculation) from the throat of a healthy carrier into the throats of healthy human beings free from diphtheria bacilli will result in the production of the carrier state and, if produced, how long it may last.

2. To determine whether the lodgment and growth of the bacilli may be prevented by the previous injection of diphtheria antitoxin.

3. To determine whether the organisms introduced are capable of producing (a) clinical diphtheria; (b) any subjective symptoms; (c) any objective signs in the appearance of the throat.

4. To determine whether any cases of clinical diphtheria develop among the associates of artificially produced "healthy" carriers of such virulent diphtheria bacilli obtained from the throat of a healthy carrier.

5. To determine whether the organisms introduced into the throats of normal human beings are in any way changed by this procedure, particularly as to (a) morphology; (b) staining characteristics; (c) cultural characteristics; (d) ability to produce toxin (virulence).

Procedure

Eight persons working in the laboratory (W. L. M., C. G. G., A. W. S., D. C. S., B. C. M., S. E. S., F. E. and W. H.) volunteered to be the subjects of these experiments. These individuals—designated for the sake of brevity in the following pages as A, B, D, V, W, X, Y and Z—were all healthy adults, varying in age from 23 to 37 years.* Three of them (A, B and D) were members of the group used in our experiments of the year before (1913), while the others (V, W, X, Y and Z) were new material. It was our original intention to include the two other persons, C and E (S. R. M. and W. A. B.), who had volunteered for the earlier series of experiments, but they were still artificially-produced carriers of non-virulent diphtheria bacilli. These two men were cultured almost daily from January 12 to May 29, 1914, but continued to be carriers throughout and so were not available for the present work. Of the eight persons who were the subjects of these experiments, two had had clinical diphtheria; D had had a severe attack at the age of five, before the introduction of antitoxin; W had had an attack at the age of six; both had recovered without the use of antitoxin. Only one had previously received antitoxin; D had received 250 units in February, 1913, almost a year before the present work was begun, as a part of the

* Five members of this group were physicians, two were medical students and one was a laboratory assistant conversant with experimental methods and especially familiar with diphtheria.

experiments undertaken at that time. None of these individuals has contracted diphtheria or received antitoxin since the close of the experiments reported here, but four of them (B, V, W and X) developed diphtheria for which they received curative doses of antitoxin in the course of this work, as will be mentioned later. Schick tests performed on these individuals showed that four (B, V, W and X) were without circulating antitoxin, as indicated by a strongly positive reaction at each trial. The tests on Y and Z were always negative, indicating some degree of immunity against diphtheria toxin. A and D showed "pseudo-positive" reactions with unheated toxin, as indicated by the occurrence of a reaction of similar character and intensity when heated toxin was used; these reactions are to be interpreted as negative and A and D are, consequently, to be grouped with Y and Z as being relatively immune against diphtheria toxin.

Preliminary throat cultures were begun on the five persons (A, B, C, D and E) used in our earlier experimental inoculations with non-virulent bacilli, on January 12, 1914. It was soon found, as was mentioned above, that C and E were still carriers of non-virulent organisms and so unavailable for the purposes of the present work. After five successive negative cultures, D showed one positive culture on January 17, containing organisms of the type with which he had been inoculated 11 months before. For this reason it was feared that he also could not be used for these experiments but the daily preliminary cultures were continued for a long period—until April 2 in the case of all members of the group except A and B—and no further positives were encountered. Additional persons were added to the group from time to time (V on January 13, W on January 18, X on January 19, Y on January 20 and Z on March 9), and preliminary cultures were continued on the entire group to ascertain whether any of the members had diphtheria bacilli in their throats at the beginning of the experiments.

For the purposes of the investigation it was planned to protect two members of the group and leave the other six without artificial protection. Accordingly Y and Z were each given a subcutaneous injection of 1000 units of diphtheria antitoxin, 24 hours prior to inoculation of their throats with virulent diphtheria bacilli.

The strain of diphtheria bacillus employed in this work (Culture 198) was obtained by us from a healthy carrier during an investigation of public school children in 1912 and, at the time of the experiments reported in the present paper (1914), it had been growing on Loeffler's blood serum for over 24 months.* When first isolated the strain proved to be virulent; repeated tests over a period of two years—20 or more, including those made at the beginning of this work—in each instance confirmed the original result. Morphologically, tinctorially and culturally the strain was typical *B. diphtheriæ*, indistinguishable from our other virulent strains and distinguishable from our non-virulent strains only by the animal test for the production of toxin.

The inoculations were made from transplants of this strain grown on Loeffler's blood serum for 24 hours and then suspended in normal salt solution. By using the growth from several tubes, this saline suspension was rendered quite thick and opaque.

The inoculations were made by soaking a cotton swab in the bacterial suspension and then rubbing it over both tonsils and the posterior pharyngeal wall. As a result of this procedure, all members of the group except Y soon showed positive cultures, so that further inoculations were considered unnecessary; by the time it became evident that Y had not developed the carrier state, our earlier enthusiasm had cooled considerably and she was not subjected to reinoculation.

As the inoculation with virulent diphtheria bacilli was believed to be a somewhat more hazardous undertaking than that carried out with non-virulent organisms in the preceding year, it was decided to try the effect of the experiment first on A and B (W. L. M. and C. G. G.). Accordingly, these two members of the group were inoculated on March 18 in the manner indicated above. Although the results of the inoculation of these two persons were known to all of the group, the other members were willing to proceed with the experiment and they were inoculated also, D, V, W, Y and Z on April 2, and X on April 3.

Daily cultures were made from the various persons engaged in the experiment from the date of inoculation until May 29 of the same year.

Results

The results of the cultures taken from January 12 to May 29, 1914, are shown in the accompanying chronological table (Table I). In this table a *positive* culture is indicated by +; a *negative* culture by 0; a day on which no culture was taken is indicated by ..; † indicates a subcutaneous injection of diphtheria antitoxin; * indicates inoculation of the throat with virulent diphtheria bacilli.

From Table I it will be seen that in all of the preliminary cultures taken prior to inoculation only one positive culture was found, that obtained from D on January 17. This culture contained non-virulent but otherwise typical diphtheria bacilli, indistinguishable from those with which his throat had been inoculated 11 months before and which were responsible for the production of the carrier state. Frequent cultures were made from D from the date of this positive culture until April 2—55, in all over a period of 75 days—and no further positives were encountered. We felt justified, therefore, in assuming that none of the eight individuals who had volunteered for these experiments were carriers of diphtheria bacilli at the time of inoculation.

On March 18, two members of the group (A and B) were inoculated by swabbing the throats with a saline suspension of virulent diphtheria bacilli obtained from the throat of a

* Concerning the child from whose throat this strain (Culture 198) was obtained, no history of diphtheria or of exposure to diphtheria previous to our investigation could be obtained; during the period covered by our observations which extended over several months, the child did not develop diphtheria nor did any case of diphtheria occur among the child's most intimate associates.

healthy carrier, as described above. The results of this procedure were strikingly different in these two individuals.

From A, who had a negative Schick test and therefore presumably had circulating antitoxin in his blood, throat cultures were negative for five days following inoculation. On the sixth day, however, his culture was positive and again on the seventeenth, the twentieth, and thirty-third day following inoculation, with 12 negative cultures between the first and last positive. After this he had 22 negative cultures in the 39 days to May 29, the end of our period of observation.

TABLE I

Day	Date	A	B	D	V	W	X	Y	Z
1	Jan. 12	0	0	0	..	..		..	..
2	Jan. 13	0	0	0	0	..	..		..
3	Jan. 14	..	0	0	0	..		..	
4	Jan. 15	0	0	0	0	..	..	..	
5	Jan. 16	0	0	0	0	..	..	..	..
6	Jan. 17	0	0	+	0	..			..
7	Jan. 18	0	0	..	0	0			
8	Jan. 19	0	0	0	0	0	0	..	
9	Jan. 20	0	0	0	0	0	0	0	
10	Jan. 21	0	0	0	0	0	..	0	..
11	Jan. 22	0	0	0		0	0	0	..
12	Jan. 23	0	0	0	0	0	0	0	..
13	Jan. 24	..	0	0	0	0	0	0	..
14	Jan. 25	0	0	0	0	0	0	..	..
15	Jan. 26		0	0	..	0	0	..	..
16	Jan. 27	0	0	..	0	0	0	0	..
17	Jan. 28	0	0	0	0	..	0	0	..
18	Jan. 29	0	0	0	..	0	0	0	..
19	Jan. 30	0	0	0	0	0	0	0	
20	Jan. 31	0	0	0	..	0	0	0	..
21	Feb. 1	0	0	..	..	0	..		
22	Feb. 2	0	0	0	0	0	0	0	..
23	Feb. 3	0	0	0	0	0	0	0	
24	Feb. 4	0	0	0	0	0	0	0	..
25	Feb. 5		0	..	..	0	..	0	
26	Feb. 6	0	0	0	0	0	0	0	
27	Feb. 7	..		..	0	0	0		
28	Feb. 8	0	0	..	0	0	..	..	
29	Feb. 9	0	0	0	0	0	0	0	
30	Feb. 10	0	0	0	0	0	0	0	..
31	Feb. 11	..	0	0	0	0		0	
32	Feb. 12	0	0	0	0	0	0	0	..
33	Feb. 13	0	0	0	..	0	0	0	
34	Feb. 14	0		0	..	0	0	0	
35	Feb. 15	..				0			
36	Feb. 16		..	..		..		..	..
37	Feb. 17	0	0	0	0	0	..	0	
38	Feb. 18	0	..	0	0	0	0		
39	Feb. 19	0	0	0	0	0	..	0	
40	Feb. 20	0	0	0	0	0	..	0	..
41	Feb. 21	0	0	0	0	0	0	0	..
42	Feb. 22	..	0	..	..	0	..		..
43	Feb. 23	..	..	..	..	..	..	..	..
44	Feb. 24	0	0	0	0	0	..	0	..
45	Feb. 25	0	0	0		0		0	..
46	Feb. 26	0	0			0	0	..	..

Day	Date	A	B	D	V	W	X	Y	Z
47	Feb. 27	0	0	0	0	0	..	0	..
48	Feb. 28	0	0	0	0	0	..	0	
49	Mar. 1	0	0	0	..	0	..	..	..
50	Mar. 2	0	0	0	0	0	..	0	..
51	Mar. 3	0	0	0	0	0	0	0	..
52	Mar. 4	0	0	0	0	0	0	0	..
53	Mar. 5	0	0	..	0	0	..	0	..
54	Mar. 6	0	..	0	0	0	0	0	..
55	Mar. 7	0	..	0			..	0	..
56	Mar. 8	0	0	0	..	0		0	..
57	Mar. 9	0	0	0	0	0	..	0	
58	Mar. 10	0	0	..	0	..	..	0	..
59	Mar. 11	0	0	0	..	0	..	0	0
60	Mar. 12	0	0	..	0	0	..	0	0
61	Mar. 13	0	0	..	0	0	..	0	0
62	Mar. 14	..	0	0	0	..	..	..	..
63	Mar. 15	..	0	0	0	0	..	..	..
64	Mar. 16	0		..	0	..	..	0	0
65	Mar. 17		0	..	0	..	..		0
66	Mar. 18	0	0	..	..	..	..	0	0
67	Mar. 19	0	-	0	0	0	..	0	0
68	Mar. 20	0	-	0	0	0	..	0	0
69	Mar. 21	0			..			0	0
70	Mar. 22	0	..	0	..	0	..	0	..
71	Mar. 23	0	..	0	0	0	0	0	0
72	Mar. 24		0	0	0	0	..	0	0
73	Mar. 25	0	+	0	..	..	0	0	0
74	Mar. 26	..	+	..	..		0	0	..
75	Mar. 27	..	0	0	0	0	0	0	0
76	Mar. 28	0	0	0	0	0	0	0	0
77	Mar. 29	..	0	0	..	..	0	0	..
78	Mar. 30	..	+	..	..	0	0	0	..
79	Mar. 31	..	+	0	0		0	0	0
80	Apr. 1	0	+	..	0	..	0	+	+
81	Apr. 2		+	0*	+	0*	0	0*	
82	Apr. 3	..	+	+		0	0*	0	0
83	Apr. 4					0	+	0	0
84	Apr. 5	0	+			..		0	
85	Apr. 6	..	..	..	..	+	..	0	0
86	Apr. 7	+	0	+		..	..	0	0
87	Apr. 8	..	+	..	..	+	..	0	0
88	Apr. 9	0	+	..	+	+	+	0	0
89	Apr. 10	0		..	..	+	0	0	+
90	Apr. 11	0	0	..	+	+	+	0	+
91	Apr. 12	0	+	..	..	..	+	..	..
92	Apr. 13	..	+		+	+	+	0	..

Day	Date	A	B	D	V	W	X	Y	Z
93	Apr. 14	..	..	+	+	..	+	..	+
94	Apr. 15	..	0	..	+	+	+	0	0
95	Apr. 16	..	0	..	+	+	+	0	0
96	Apr. 17	0	+	+	+	+	+	0	+
97	Apr. 18	..	..	..	+	+	0	..	0
98	Apr. 19	..	0	..	..	+	+	0	+
99	Apr. 20	+	..	..	..	+	+	0	0
100	Apr. 21	0		..	+	+	+	0	+
101	Apr. 22	0	0	+	+	+	+	0	+
102	Apr. 23	0	0		..	+	+	0	0
103	Apr. 24	0	..	..	+	+	+	0	+
104	Apr. 25	..	0	+	+	+	+	0	..
105	Apr. 26	0	0	+	+	..	..	0	+
106	Apr. 27	..	..	..	..	..	..	..	0
107	Apr. 28	0	0	..	..	+	+	0	+
108	Apr. 29	..	0	+	..	+	..	..	0
109	Apr. 30	0	0	+	+	0	+	0	+
110	May 1	0	0	0	+	+	+	0	..
111	May 2	..	+	+	+	+	+	..	..
112	May 3	0	..	..	..	..	+	0	..
113	May 4	0	+	+	+	..	0	0	+
114	May 5	..	0	+	0	+	+	..	..
115	May 6	0	..	..	+	+	+	..	..
116	May 7	..	+		+	+	+	..	+
117	May 8	..	..	0	+	+	+	0	0
118	May 9		..	0	+	+	+	0	+
119	May 10	0	0	0	+		..	0	..
120	May 11	..	0	+	+	+	..	0	+
121	May 12	..	0	..	+	..	+	..	0
122	May 13	..				+	+	0	0
123	May 14	0		+	+	+	+	..	..
124	May 15	0					..	+	0
125	May 16	0					+		+
126	May 17		..	..	..		+	0	..
127	May 18			..	..	+	+	..	0
128	May 19						+	0	0
129	May 20	..	0			..	+	0	+
130	May 21	0		..	..	+	+	0	+
131	May 22	..	0		..	+	0	..	0
132	May 23	..	0	..	..	+	..	0	..
133	May 24	..	..	..	..		..	..	..
134	May 25	0	0	..		+	0	+	+
135	May 26	0	+	..	+	+	..	..	+
136	May 27	..	0	..		+		..	+
137	May 28	..		..	+	..	..	..	..
138	May 29	0	+	..	+	0			

B, who at every test showed a strongly positive Schick reaction and therefore was presumably without natural immunity against diphtheria toxin, developed a frank attack of clinical diphtheria, following which he continued to harbor diphtheria bacilli and was still a carrier at the time of our last examination on May 29. From the date of inoculation he had 27 positive and 30 negative cultures over a period of 72 days.

On April 1, two members of the group (Y and Z) received 1000 units of diphtheria antitoxin subcutaneously. On the following day these two, together with three other members (D, V and W), who received no antitoxin, were inoculated by swabbing the throats with a saline suspension of virulent diphtheria bacilli, as described above. X, the remaining member of the group who also had received no preliminary injection of antitoxin, was inoculated in like manner on the following day, April 3. Here again the results of inoculation differed markedly in the various individuals.

D, who had received no antitoxin but who had a negative Schick test, did not develop clinical diphtheria but promptly became a carrier and remained a carrier to the last observation on May 22. During this time (50 days) there were obtained from him 21 positive and 5 negative cultures.

V, W and X who had received no antitoxin but each of whom had a positive Schick test, all developed diphtheria, following which they all became carriers and continued to habor diphtheria bacilli practically to the end of the period of observation on May 29. Following inoculation, V had 37 positive cultures and 1 negative culture; W had 41 positive and 4 negative; X had 36 positive and 7 negative.

Y and Z, each of whom had received a preliminary injection of antitoxin and who already had some degree of natural antitoxic immunity as manifested by a negative Schick test, did not develop diphtheria. The only evidence, and this must be regarded as inconclusive, that Y became a carrier, was the one positive culture which occurred 43 days after inoculation and 15 days before the last observation on May 29. Following inoculation she showed, in all, 1 positive culture and 39 negative cultures in the 57 days which elapsed to the date of her last culture on May 29. Z showed his first positive culture on the third day after inoculation. This was followed by 4 negative cultures, then came 4 positives, after which his cultures were irregularly positive and negative. He was still a carrier at our last observation on May 27. Following inoculation he showed in all 22 positive and 20 negative cultures.

It is seen, therefore, that as a result of experimental inoculation with virulent diphtheria bacilli originally obtained from the throat of a healthy carrier, the carrier state was established in seven out of eight members of the group; in one who had received a preliminary injection of antitoxin (Z) and in six who had not (A, B, D, V, W and X); in four who gave a positive reaction to the Schick test (B, V, W and X), and in three whose Schick test was negative (A, D and Z). The remaining member of the group (Y) failed to develop the carrier state and showed only one, isolated, positive culture 43 days after inoculation. This person had a negative Schick test and had also received a preliminary injection of antitoxin.

The results obtained in the course of our observations (Table I) are summarized in Tables II and III.

TABLE II

Date of Event	A	B	D	V	W	X	Y	Z
First preliminary culture	Jan. 12	Jan. 12	Jan. 12	Jan. 13	Jan. 18	Jan. 19	Jan. 20	Mar. 9
Last preliminary culture	Mar. 16	Mar. 17	Apr. 2	Apr. 1	Apr. 2	Apr. 3	Apr. 2	Mar. 31
Preliminary injection of antitoxin	......	......	......	......	......	......	Apr. 1	Apr. 1
Inoculation with *B. diphtheriæ*	Mar. 18	Mar. 18	Apr. 2	Apr. 2	Apr. 2	Apr. 3	Apr. 2	Apr. 2
First curative injection of antitoxin	......	Mar. 20	......	Apr. 5	Apr. 6	Apr. 5	......	......
First positive culture	Mar. 24	Mar. 19	Apr. 3	Apr. 3	Apr. 5	Apr. 4	May 15	Apr. 5
Last positive culture	Apr. 20	May 29	May 22	May 29	May 27	May 21	May 15	May 27
Last culture taken	May 29	May 29	May 22	May 29	May 29	May 29	May 29	May 27

TABLE III

Cultures	A	B	D	V	W	X	Y	Z
Total	96	115	87	95	108	83	100	58
Preliminary	53	58	61	57	63	40	60	16
After inoculation with *B. diphtheriæ*	43	57	26	38	45	43	40	42
After inoculation before first positive	5	0	0	0	2	0	31	2
Total positive	4	27	21	37	41	36	1	22
Total negative	39	30	5	1	4	7	39	20
Per cent positive	9.30	47.36	80.76	97.36	91.11	83.72	2.50	52 38

From Tables I, II and III it is seen that even the approximate time of persistence of diphtheria bacilli in the throats of these eight persons can be judged only in regard to two members of the group, A and Y. A showed only 4 positive cultures, the first 6 days and the last 33 days after inoculation. As the last positive was followed by 22 negative cultures over a period of 39 days, we may assume that A actually harbored diphtheria bacilli for at least 28 days and very likely for 33 days, since the organisms were probably present from the date of inoculation although not recovered until 6 days later. Inasmuch as Y showed only one positive culture which occurred 43 days after inoculation and this was followed by 8 negative cultures in the remaining fortnight, there is no evidence other than inferential to show that she harbored diphtheria organisms longer than one day.

Concerning B, D, V and Z, from each of whom a positive culture was obtained at the last observation, it is evident that they were carriers at least until that date and may readily have continued to be carriers for a long time thereafter. The known period during which they harbored diphtheria bacilli was, therefore, in the case of B, 72 days; for D, 50 days; for V, 57 days; and for Z, 53 days.

Neither W nor X showed a positive culture at the last examination, so we cannot be sure that either continued to harbor diphtheria bacilli after that date, but it is extremely probable that at least one and possibly both of them were carriers for a longer time than our results actually indicate. From Table I it is seen that although the last culture from W was negative, the 21 which preceded it were positive and that, of 43 cultures taken after diphtheria bacilli were first recovered from this member of the group, only 2 cultures were negative. Our evidence shows that diphtheria bacilli were present in the throat of W for at least 53 days and in that of X for at least 48 days.

It is obvious that the previous administration of 1000 units of diphtheria antitoxin did not prevent the lodgment and growth of the virulent diphtheria bacilli in the throat of Z. We cannot be perfectly sure that it did not prevent their colonization in the throat of Y; it should be pointed out, however, that although Y had only one positive culture, A, who received no preliminary injection of antitoxin, had only 4 positive cultures, so the preventive effect, if any, is not striking.

Likewise, the presence of natural immunity against diphtheria toxin did not prevent the development of the carrier state in A, D and Z, although Y, who also possessed natural antitoxic immunity as evidenced by a negative Schick test, showed only one positive culture.

The value of antitoxic immunity, natural or artificial, becomes quite apparent, however, when we come to consider the effect of the inoculation with virulent organisms obtained from the throat of a healthy carrier upon the health of these eight individuals. All of those with positive Schick tests (B, V, W and X) developed diphtheria; none of those with negative Schick tests (A, D, Y and Z) contracted the disease. Y and Z each had their natural immunity reinforced by a preliminary injection of antitoxin, so that they were spectators of, rather than participants in, whatever excitement was attendant upon the experiments.

The diphtheria from which the four less fortunate members of the group (B, V, W and X) suffered was very real, very definite, but its extent in V, W and X was wholly unpremeditated. Following inoculation of the throats of A and B on March 18, A suffered no ill effects whatever and his cultures did not become positive for 6 days, but events developed rapidly in the case of B. On the day following inoculation his culture was positive, that afternoon his throat became very sore, so much so that by suppertime he could scarcely swallow and talking became difficult. Before bedtime he had several severe chills and by the next day there was a definite diphtheritic membrane on both tonsils and extending over the soft palate toward the uvula. His temperature meanwhile rose to 103° F. and he suffered from severe headache and backache. That no question might arise as to the objective appearance of the throat, a laryngologist was consulted, who promptly made a diagnosis of diphtheria before any of the details concerning the experiment were communicated to him. B was then given a subcutaneous injection of 5000 units of diphtheria antitoxin, followed by the injection of a similar amount on the succeeding day. The membrane rapidly disappeared and the disease cleared up promptly without complications or sequelæ that were noted.

This experience served as a sharp warning of what might be expected on inoculation of susceptible individuals with virulent diphtheria bacilli from the throat of a healthy carrier, and for a time markedly diminished our zeal for further experimentation of this sort. After the lapse of a fortnight, however, our enthusiasm was partially regained and it was decided to carry out the experiment with the other members of the group. Each of them knew the result of the previous inoculations and the risks were freely discussed, but all were willing to proceed with the experiment. Accordingly, Y and Z each received a subcutaneous injection of 1000 units of antitoxin on April 1, and the following day these two, together with D, V and W, were inoculated in the same manner as that used for A and B. X was inoculated a day later. All received instructions to report at the first appearance of symptoms, as it was desired merely to test their susceptibility and to avert frank attacks of diphtheria by prompt administration of antitoxin. The results have been referred to above.

Y and Z, each of whom had a negative Schick test and had also received a preliminary injection of antitoxin, showed no ill effects following inoculation. One became a carrier, the other did not.

D, who received no preliminary injection of antitoxin but who had a negative Schick test, became a carrier, but presented no other subjective or objective effect of inoculation.

Notwithstanding the explicit instructions given, V, W and X did not report at the earliest appearance of symptoms, but only when these had become outspoken. Each of the three had a definite attack of clinical diphtheria with false membrane formation. The severity of the disease differed some-

what, W having the least and X the most severe reaction, while the intensity of the attack in V was between these two extremes. The manifestations in all three followed the same course as in B, the incubation period being practically identical, the constitutional symptoms similar and the fever outspoken in all, W reaching a maximum of 102° F., V of 102.6° F. and X of 104.7° F. Each recovered promptly following the administration of antitoxin and all became carriers.

No cases of clinical diphtheria developed among the associates of the eight members of this group.

To determine whether any change had taken place in the bacilli as the result of their residence in the human throat, the organisms were isolated at intervals from the positive cultures obtained from the various members of the group and tested culturally, tinctorially and by guinea-pig inoculation. Morphologically, the organisms were unchanged and their staining characteristics were the same as before they were introduced into the throat. On cultural tests, also, no difference was observed in the character of the growth on Loeffler's blood serum, plain agar or in bouillon, while the changes in litmus milk and in the test for fermentation of the various sugars were the same as with the original organisms. Many of these pure cultures were tested for virulence. In every instance the result was the same as with the original strain used for inoculation. There was, therefore, no evidence of loss of virulence in the bacilli as the result of their residence in the human throat.

The question of the identity of the organisms recovered from the positive cultures with the strain used for inoculation needs little discussion. The eight individuals in this group were shown to be free from diphtheria bacilli by a long series of preliminary cultures; they were then inoculated and all but one became carriers, either healthy or convalescent. The organisms which they harbored agreed with the original strain in morphology, cultural reactions and virulence.

SUMMARY

1. *Will the introduction into the throats of healthy human beings of virulent diphtheria bacilli from the throat of a healthy carrier result in the production of the carrier state and, if produced, how long may it last?*

The carrier state was produced in seven out of eight individuals by a single inoculation of the throat with virulent diphtheria bacilli obtained from the throat of a healthy carrier. The remaining member of the group showed only one positive culture which occurred 43 days after the date of inoculation. Four of these seven individuals developed clinical diphtheria and, since they continued to harbor organisms after recovery from the disease, are to be considered as convalescent carriers. The other three carriers did not contract clinical diphtheria and so may be termed healthy carriers.

The actual duration of the carrier state in any of the seven persons was not determined. It was at least 28 days in the case of A, 72 days in the case of B, 50 days in D, 57 days in V, 53 days in W, 48 days in X, and 53 days in Z. These figures are based upon the time elapsing between the date of the first positive culture and the last positive culture. Inasmuch as each of these persons probably harbored diphtheria organisms from the time of inoculation, although three of them did not show positive cultures until a few days later, it would be more nearly correct to increase the minimal period by 5 days in the case of A, and 2 days each for W and Z. Four of the seven carriers (B, D, V and Z) showed a positive culture at the time of the last examination, so that we feel reasonably sure that they continued to be carriers after that date. Two others (W and X) did not show positive cultures at the last examination, but had done so practically constantly up to that time. It is less certain but nevertheless quite probable that the last positive cultures obtained from these two persons did not measure accurately the duration of the carrier state. A was the only carrier concerning whom we feel assured that he no longer harbored diphtheria organisms at the termination of our period of observation. The probable duration of the carrier state in these seven individuals, therefore, may be tentatively estimated as follows: A 33 days, B 72 days plus, D 50 days plus, V 57 days plus, W 57 days plus, X 56 days plus and Z 55 days plus.

2. *May the lodgment and growth of the bacilli be prevented by the previous injection of diphtheria antitoxin?*

The lodgment and growth of the organisms introduced into the throat of Z were not prevented by the previous injection of antitoxin. In Y, who showed only one positive culture and that 43 days after inoculation, the development of the carrier state may have been prevented by the antitoxin which she received the day prior to inoculation. It has been pointed out, however, that A, who received no preliminary injection of antitoxin, showed only 4 positive cultures. The effect of diphtheria antitoxin, therefore, in preventing the colonization of the organisms introduced, was neither complete nor striking; from the results of our previous experiments with animals * and with human beings we think that it probably had no influence whatever in preventing the development of the carrier state.

Natural immunity against diphtheria toxin as manifested by a negative Schick test did not prevent the development of the carrier state in A, D and Z, but Y, who received a preliminary injection of antitoxin and whose skin test was also negative, showed only one positive culture. The evidence is, therefore, far from convincing that the presence of natural immunity against diphtheria toxin may prevent the lodgment and growth of diphtheria bacilli in the throat.

3. *Are such organisms, when introduced, capable of producing (a) clinical diphtheria; (b) any subjective symptoms; (c) any objective signs in the appearance of the throat?*

The four members of the group (A, D, Y and Z), who gave evidence of natural immunity against diphtheria toxin, as evidenced by a negative Schick test, two of whom (Y and Z) had this natural immunity reinforced by a preliminary injec-

* Gelien, J., Moss, W. L., and Guthrie, C. G.: The Effect of Diphtheria Antitoxin in Preventing Lodgment and Growth of the Diphtheria Bacillus in the Nasal Passages of Animals. Johns Hopkins Hosp. Bull., 1920, XXXI, 381.

tion of antitoxin, did not develop clinical diphtheria and showed no local or general effect, either objective or subjective, as the result of inoculation with virulent diphtheria bacilli from the throat of a healthy carrier.

The four persons (B, V, W and X), who had no natural antitoxic immunity as evidenced by a positive Schick test and who received no preliminary injection of antitoxin, all developed definite clinical diphtheria. From these four cases, however, we cannot say that the same result would invariably follow the introduction of virulent organisms into the throats of persons giving a positive Schick test.

4. *Will any cases of clinical diphtheria develop among the associates of artificially produced, "healthy" carriers of virulent diphtheria bacilli obtained from the throat of a healthy carrier?*

As has been pointed out, two sorts of carriers were produced among the persons in the group by the inoculation with virulent diphtheria bacilli. No cases of clinical diphtheria developed among the associates either of the healthy carriers (A, D and Z), or of the convalescent carriers (B, V, W and X).

5. *Are the organisms introduced into the throats of normal human beings in any way changed by this procedure, particularly as to (a) morphology; (b) staining characteristics; (c) cultural characteristics; (d) ability to produce toxin (virulence)?*

When isolated in pure culture after prolonged sojourn in the human throat, the bacilli were not altered in morphology or in their staining or cultural characteristics. The bacilli recovered from each of these persons throughout the period covered by our observations had retained their ability to produce toxin.

Comment

From the results of these experiments it is evident that there is no essential difference between virulent diphtheria bacilli (as determined by guinea-pig inoculation) from the throat of a healthy carrier and those from a patient with clinical diphtheria. The reliability of the animal test for toxin production has been confirmed, since it has been shown that organisms which are virulent for guinea-pigs are capable of producing diphtheria in susceptible human beings. Our earlier conclusions concerning the harmless nature of non-virulent diphtheria bacilli have received indirect confirmation since one member of the group (B), who became a carrier but suffered no other ill effect, subjective or objective, as the result of the introduction of non-virulent organisms into his throat in 1913, promptly developed clinical diphtheria following inoculation with virulent organisms in 1914. The reliability of the Schick reaction as an index of antitoxic immunity is well shown by the fact that all members of the group with a positive skin test developed diphtheria following inoculation, whereas those with a negative skin test did not.

Two questions were mentioned in the introduction which are not answered by our work. Evidently the failure of healthy carriers of virulent diphtheria bacilli to contract the disease is not because the organisms which they harbor are incapable of producing it. May this immunity be dependent upon the presence of circulating antitoxin? Information on this point is scanty, as those authors who refer to the subject at all have, for the most part, made only general statements, drawing no distinction between convalescent and healthy carriers or failing to mention whether the carriers harbored virulent or non-virulent organisms. Our own experience has not been extensive but is given, together with that of a few others, in the following table (Table IV).

TABLE IV

Results of Schick Tests

Observer	In convalescent carriers of virulent organisms	In healthy carriers of non-virulent organisms	In healthy carriers of virulent organisms	After spontaneous recovery from diphtheria
Kolmer*	Pos. or neg.	Pos. or neg.	Negative.	....
Park †	Pos. or neg.	Pos. or neg.	? [3]	Pos. or neg.[1]
Place ‡	Pos. or neg.[2]	Pos. or neg.	Negative.	Pos. or neg.[5]
Guthrie, Marshall and Moss.	Pos. or neg.	Pos. or neg.	Negative.	Pos. or neg.

* Kolmer, John A., and Moshage, Emily L.: The Schick Toxin Reaction for Immunity in Diphtheria, Am. J. Dis. Child., 1915, IX, 189.
† Park, W. H.: Personal communication.
‡ Place, E. H.: Personal communication.

[1] Usually become negative within a few months after they were positive at first.
[2] Almost invariably negative but occasionally positive and in such cases the patient usually develops diphtheria again.
[3] Data insufficient but Park says he knows that a few are Schick-positive.
[4] Majority of seven cases Schick-positive.
[5] Negative immediately after recovery but may become positive later.

From Table IV, column 4, it is apparent that in many carriers of virulent diphtheria bacilli, failure to develop the disease may reasonably be attributed to adequate protection by circulating antitoxin, since they give a negative response to the Schick test. Although we have encountered no exception to this rule in our own work, the results of Park suggest that some of the healthy carriers of virulent organisms are without protection by circulating antitoxin; we feel, therefore, that a sweeping conclusion from our own small number of observations might be misleading. There may be other factors which play a part in the immunity which these carriers possess; at least such a possibility merits consideration.

Certain rather puzzling facts are known which serve to accentuate the incompleteness of our knowledge concerning diphtheria.

(1) Convalescent carriers may harbor virulent organisms for long periods after recovery from the disease, long after all passive immunity from injected antitoxin has disappeared, and show strongly positive skin tests indicating an absence of active immunity derived from circulating antitoxin developed by the carrier (Table IV, column 2).

(2) It has long been believed that individuals recovering from diphtheria without antitoxin treatment, accomplished this feat by means of the development of an active immunity, and this view is probably correct. We had supposed that this immunity was due to the elaboration of antitoxin within the body of the patient, and so were greatly surprised to learn that in persons recovering from diphtheria spontaneously, the Schick test may be positive, indicating an absence of sufficient

circulating antitoxin to prevent the recurrence of the disease (Table IV, column 5). As has been mentioned, one member of our group (W) had diphtheria at the age of six, recovered without treatment with antitoxin and showed a positive Schick test years later; that this was a true indication of susceptibility to diphtheria was proven by the fact that she developed the disease following experimental inoculation of her throat.

(3) Although it is believed that individuals possessing a natural antitoxic immunity, as manifested by a negative Schick test, are not susceptible to diphtheria, by no means all persons with a positive Schick test contract the disease even on prolonged and repeated exposure. Persons with positive Schick tests differ markedly in this respect, some never contract the disease and others have repeated attacks; so pronounced is this difference that it cannot be entirely due to a difference in exposure to infection. Another member of our group (B) was frequently exposed to diphtheria for several years, in the care of patients ill of the disease, in the study of carriers and in experimental work in the laboratory. It may be urged that there is no proof that diphtheria bacilli actually reached the nose or throat of this individual in the course of any of these procedures, but no one with much experience in taking throat cultures from children has escaped plentiful spraying from his patients. In the experimental work with animals, virulent diphtheria bacilli were introduced into the nostrils of cats, rabbits and guinea-pigs by means of a tiny swab made of a wisp of cotton on the end of a pig bristle. During the course of this work which was carried on at close range, the animals responded to each introduction of the swab with vigorous sneezing which can scarcely have failed to disseminate the bacilli and "expose" B rather thoroughly. His Schick test was positive, yet his only attack of diphtheria followed experimental inoculation with a massive dose of virulent organisms in the course of the work which forms the basis of this paper.

(4) It has been shown that the introduction of virulent diphtheria bacilli into the upper respiratory passages of several species of animals will not cause diphtheria, although the animals are susceptible to the effects of diphtheria toxin when it is injected. If the inoculation is accompanied by abrasion of the mucous membrane, however, diphtheria may develop promptly. Other paradoxical observations might be added to the list but these will suffice.

How are these apparently conflicting facts to be reconciled with each other and with the existing views concerning the immunity in diphtheria? The positive Schick test, which undoubtedly occurs sometimes in persons who recover from diphtheria without treatment with antitoxin, may possibly be explained as follows: An individual, recovering spontaneously, may do so as the result of an active immunity resulting from the elaboration of his own antitoxin. If he produces it readily and in great excess he would probably remain Schick-negative for life and not again contract the disease. He might produce it much less generously—just sufficiently to overcome the disease—have a slight excess present for only a short time after the attack and eventually show none at all. Such a person presenting a positive Schick test before the attack, would probably show a negative reaction immediately thereafter but somewhat later would again become positive. Thus, an individual after spontaneous recovery from diphtheria might present either a positive or a negative reaction, depending to a certain extent on when the test was made.

The other points mentioned—(1), (3) and (4)—are more difficult to reconcile and suggest that there may be more than one kind of immunity in diphtheria. The assumption of a local, cellular immunity seems necessary to explain the conditions mentioned above concerning diphtheria in animals. Just as animals quite susceptible to the effect of diphtheria toxin, when injected, differ from susceptible human beings in the degree of resistance of their mucous membranes, so susceptible human beings (positive Schick test) may differ from each other in the same respect. Such an immunity would go far to explain why those convalescent carriers who have a positive Schick test do not invariably and immediately have another attack. It would also offer a plausible explanation for the observation that some individuals with a positive Schick test may resist prolonged exposure to infection. If some of the healthy carriers of virulent diphtheria bacilli should be found to have positive Schick tests, it would explain their failure to develop the disease.

We are unable to say why it has been so difficult to trace cases of clinical diphtheria to healthy carriers of virulent diphtheria bacilli. As has been pointed out, it is certainly not because the organisms which they harbor are incapable of causing the disease nor can it be dependent upon natural immunity possessed by all their associates, if the immunity be limited to the presence of circulating antitoxin. Here also, the presence of a local, cellular immunity in some of the persons apparently susceptible would help to explain the facts observed. This is merely another way of saying that the number of persons in the community who are relatively insusceptible to diphtheria may be much greater than would be surmised from the results of the Schick test alone. If such an immunity could be shown to occur, it would help materially to explain the great discrepancy which exists between the number of carriers of virulent organisms and the incidence of cases of clinical diphtheria, by emphasizing the relatively slight opportunities for doing harm open to the healthy carrier of virulent diphtheria bacilli.

Above all, we would not be interpreted as attempting to question the well-established fact of antitoxic immunity or to discredit the Schick test which is a most valuable index of such immunity. It is not impossible to reconcile the facts observed with the theory advanced. The relation between toxin and antitoxin is a quantitative relation. When properly performed a negative Schick test indicates an antitoxin content of at least 1/40 to 1/30 of a unit per cubic centimeter of blood. No one thinks for a moment that, if an individual with a negative Schick test were subjected to a very large injection of toxin of high potency, his natural immunity would enable him to escape all harmful effect. In a similar way, the local defense might be overcome by the introduction of an

enormous number of organisms at one time, particularly if these were rubbed in, as was the case in our inoculations. We may carry the analogy further. By no means everyone has natural antitoxic immunity; it is quite improbable that all have any considerable local immunity; theoretically, an individual might have either, neither or both. Each type, when present, probably confers relative rather than absolute protection upon the possessor. It is possible that none of the four members of our group with positive Schick tests, who developed diphtheria following inoculation, possessed any local immunity, but if they had, one could understand how this outer defense was overwhelmed by the method of attack. All this is mere theory, however, in itself unprofitable unless followed up by experimental proof.

In a few words may be given our impression concerning the relation of the healthy carrier of virulent diphtheria bacilli to the health of the community. Although the results of our experiments indicate that the virulent organisms from the throats of healthy carriers are capable of causing clinical diphtheria when the proper opportunity is afforded, we have not obtained evidence, either from this work or from our previous studies of carriers, that diphtheria is spread by their agency under ordinary conditions. It is our belief (1) that such carriers constitute a potential menace to the health of the community but (2) that their opportunity for dissemination of the disease among their associates is quite limited owing to the relatively small number of persons who are very susceptible to infection and consequently (3) that the actual part which they play in the spread of diphtheria is probably quite small.

Conclusions

1. Virulent diphtheria bacilli present in the throats of healthy carriers are capable of producing clinical diphtheria and do not differ from those obtained from patients with the disease.

2. Virulent diphtheria bacilli retain their characteristics despite long residence in the human throat or transfer from one human being to another.

3. The guinea-pig test is a reliable index of the inherent ability of diphtheria bacilli to cause clinical diphtheria in susceptible human beings.

4. The Schick test is a reliable index of the presence or absence of antitoxic immunity against diphtheria.

5. Experimental diphtheria in human beings has a short incubation period, produces marked constitutional effects, and is accompanied by a sharp febrile reaction. It may be cured promptly by the early injection of antitoxin in adequate dosage.

THE SIGNIFICANCE OF "HEMOLYTIC INFLUENZA BACILLI"

By Arthur L. Bloomfield

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As the study of respiratory bacteriology has progressed, it has become apparent that special explanations are needed for the presence of various organisms in the upper air passages and that it is no longer possible to assume that the chance advent of a bacterium is followed by colonization and growth.[1] In another place[2] we have attempted to define the significance of the various bacteria encountered in the upper respiratory tract according to the following scheme:

A. The organism may be a member of the constant normal throat flora.

B. The organism may be an extraneous one which is present as a transient in the throat.

C. The organism may have become established in a local focus of disease in the upper air passages.

D. The organism may be one which is the cause of and is associated with the presence of an acute disease.

But aside from the above, still another possible explanation of the presence of bacteria in the upper air passages presents itself, namely, that under special conditions an organism may attain a more or less wide dissemination among groups of individuals without being primarily the cause of disease and without being localized in a definite focus of infection. Certain observations, such as the frequent presence of hemolytic streptococci[3] or meningococci[4] in the throats of contacts during epidemics of disease caused by these organisms, suggest that this may be the case, although complete demonstration of such a state of affairs is still lacking.

During the spring of 1920, while making studies of the throat flora of healthy individuals,[5] we were impressed by the almost constant finding of influenza bacilli in large numbers in practically all of these people. At the time we were inclined to regard these organisms as normal inhabitants of the pharynx, but inasmuch as there had recently been an epidemic of respiratory disease (influenza), it seemed wiser to delay judgment of their exact status. After an intermission during the summer, work was resumed in the fall of 1920 and we were surprised to find the influenza bacillus conspicuously absent in cultures made by exactly the same methods in a similar and to some extent identical group of individuals. On the other hand, a hemolytic Gram-negative hemophilic bacillus which was encountered in only two cultures in the spring of 1920 was found with almost constant uniformity in the throats both of normal people and those suffering from "colds." This observation seemed of sufficient interest to warrant following it further, inasmuch as light might thereby be thrown on general questions of the significance of bacteria found in the throat under various conditions.

We wish, therefore, to present some observations on the occurrence of "hemolytic influenza bacilli," not with the idea of contributing anything new as to the general incidence of

this organism in the upper air passages, but in order to bring out its significance in relation to that of other groups of bacteria.

THE ORGANISM

The hemolytic influenza bacillus, as studied by us, is culturally and morphologically essentially identical with the influenza bacillus save that, when grown on blood agar, a wide zone of hemolysis of the so-called beta type is produced. The colonies on this medium vary in size up to 2 mm.; they are pale greyish, rather translucent, and usually slightly sticky. The organisms are Gram-negative, and smears from 24-hour growths show long and short bacilli or thread forms. Growth is scant on human blood agar, absent in ordinary media not containing hemoglobin, and profuse on Avery's medium. This organism was described in 1919 under the name of Bacillus X by Pritchett and Stillman, whose original account may be quoted.[6] "The colony varied between 1 and 2 mm. in diameter, was clear, convex and mucoid. . . . . The bacillus is Gram-negative, takes the counter-stain deeply, and when taken from agar surface cultures appears as long, tangled threads somewhat similar to the threads often seen in pure cultures of Bacillus influenzæ. In blood-broth cultures it appears as a small fat bacillus without chain formation. It does not grow on plain sheep serum, or glucose agar.

"On blood agar the abundant growth appears as a somewhat granular, fairly heavy film, with marked hemolysis of red cells. This hemolysis is as marked as that produced by hemolytic streptococci. . . . . No pathogenicity could be demonstrated for mice, rats or rabbits." This organism was encountered by Pritchett and Stillman "in about two dozen individuals" during a series of cultures made on several hundred healthy people. They make no comment on its significance, but apparently do not regard it as etiologically related to respiratory infection. During some of the epidemics of measles in the military camps this organism was commonly present,[7] and various observers have found it from time to time in the throats of normal people. Rivers[8] reports its presence in about 30 per cent of normal men, and in a series of about 100 cultures made in the spring of 1920 we isolated it twice.

In summary, then, these hemolytic Gram-negative hemophiles, according to previous observations, are not members of the constant normal throat flora, but do occur from time to time in healthy individuals. They are non-pathogenic for animals and do not appear to be causally related to disease in man.

METHODS

A swab passed systematically over the tonsils, pharynx, and soft palate was plated on rabbit blood agar. A technique was employed which secured a good spread of colonies. The organisms were identified by colony appearance, morphology, and subculture. The number per swab was estimated as accurately as possible.

The individuals studied comprised a heterogeneous group of physicians, nurses, medical students, laboratory attendants, and hospital patients. In some cases several cultures were made, in others only a single one. "Colds" were prevalent during part of the period covered by the study and some of the subjects were affected at the time at which the cultures were obtained.

The results are summarized in the following tables:

TABLE I

INCIDENCE OF "HEMOLYTIC INFLUENZA BACILLI" IN THROAT CULTURES FROM NOVEMBER, 1920, TO AUGUST, 1921

Nov. 1–Nov. 15. .	+++++++	
Nov. 16–Nov. 30	++++++++++++ ++++	0
Dec. 1–Dec. 15. . .	+++++++++++	000
Dec. 16–Dec. 31. .	+++	000
Jan. 1–Jan. 15 . .	++++++++++++ ++	00000000000000
Jan. 16-Feb. 1 . .	+++++++++++	0000000000000000000 000000000000
March	++++++	0000000
April	++++++++	0000000000
May	+++	0000
August	++++++++++++ +++++	0000000000000000000 000000

* Each positive culture is indicated by (+) and each negative by (0)

TABLE II

NUMERICAL SUMMARY OF TABLE I

Date	Total No. cultures	Per cent positive	Total No. of people examined	Per cent positive
Nov. 1–Nov. 15	6	100	3	100
Nov. 15–Dec. 1	17	94	7	100
Dec. 1–Dec. 15.	14	80	5	80
Dec. 15–Jan. 1.	6	50	4	50
Jan. 1–Jan. 15.	28	50	23	48
Jan. 15–Feb. 1.	42	36	35	40
March	13	49	4	100
April	18	44	9	66
May	7	47	5	60
August.	52	33	52	33

From Tables I and II it appears that hemolytic influenza bacilli were found almost uniformly in cultures made during November and December, 1920, whereas, since then, the incidence has been somewhat below 50 per cent. That the latter figure has been more or less constant for the past six months seems to indicate the present normal level. In looking for an explanation for the higher figure during the earlier cultures a possible but uncertain explanation is found in the fact that "colds" were especially prevalent at that time and that most of the cultures were made during or following an acute coryza. On the other hand, in several cases of "colds" hemolytic influenza bacilli were altogether absent.

In a further attempt to explain the presence of these organisms a series of differential cultures was made from various parts of the upper air passages. The results are summarized in Table III. It appears that in every case but one the organisms were present only sporadically and in small numbers. In Ma, on the other hand, they were constantly present in large numbers on the pharynx and both tonsils. This person had had no clinical respiratory infection during the past winter and showed no special abnormality in the upper air passages. The tonsils were small and scarred.

Discussion

Before discussing the significance of the facts brought out in the tables in regard to the occurrence of "hemolytic influenza bacilli," it is advisable to summarize certain general points. In the first place, it does not seem possible to attach any pathological significance to the presence of these organisms, at least during the past winter. The majority of individuals harboring them were clinically normal. While they were frequently present in some cases of "colds," in other instances they were persistently absent. Furthermore, the topographical cultures did not show, except in the case of Ma, a predilection for any particular part of the upper air passages—the organism was isolated from tongue, tonsils, or pharynx. In other words, a carrier state in association with a local focus of diseased tissue, such as a tonsil, cannot be assumed as a general explanation of its presence. Finally, we are dealing with an organism which is perishable outside the body and which is not an inhabitant of the skin surfaces. There is no apparent source, therefore, which would account for a frequent transient invasion as in the case of Staphylococcus albus which is readily introduced from the nose or body surface.

What then is the explanation of the presence of these organisms in about 50 per cent of a large series of throat cultures? As pointed out elsewhere,[5] there is evidence to show that certain bacteria have become adapted to a free growth on the mucous membranes of the upper air passages. It is found for example that the green-producing streptococcus, which is a member of the normal flora of adult throats, is already established in the mouths of infants 24 hours after birth. A high degree of affinity must exist here, which perhaps has developed during a long period of evolution. On the other hand, there exist bacteria which under all circumstances seem to fail to adapt themselves to growth in the upper air passages. The colon bacillus, for example, despite its ready access to the mouth is not found there in the case of clinically normal individuals. Is it possible that under special and as yet undefinable circumstances partial, abortive, or temporary adaptations of organisms to growth in the upper air passages may occur? Such an assumption would account for the wide occurrence of influenza bacilli at the time of the epidemic of 1918 and during the year following it, and for the reported instances of large numbers of temporary carriers of hemolytic streptococci and meningococci in association with epidemics of disease due to these organisms. We have also collected numerous individual observations which can be explained in no other way. Er, for example, whose throat flora had been studied at frequent intervals for over a year suddenly yielded a yeast in large numbers in the cultures. This organism had not previously been present, and there was no disease or infection at

TABLE III

Differential Cultures from Various Parts of the Upper Air Passages Showing Incidence of Hemolytic Influenza Bacilli

Patient	F.							Bl.						Er.			Ma.				
Date	Mar. 12	Mar. 14	Mar. 16	Mar. 1[illegible]	Mar. 2[illegible]	Apr. 12	May 1[illegible]	Mar. 1[illegible]	Mar. 22	Mar. 2[illegible]	Apr. 11	May 15	Mar. 21	Mar. 31	Apr. 11	Apr. 2[illegible]	Mar. 22	Apr. 4	Apr. 16	May 3	May 16
Nose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pharynx	0	0	0	0	0	0	many	0	0	0	0	0	0	0	0	0	several hundred	40	a few	x	many
Right tonsil	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	many	x	x
Left tonsil	0	0	0	0	0	0	0	0	0	0	0	0	0	a few	0	0	many	1	many	many	60
Tongue	0	0	0	0	0	20	40	2	0	50	0	0	0	0	0	3	0	0	0	0	0

Patient	H.						D.				B.			M.		
Date	Apr. [illegible]	Apr. 12	Apr. 1[illegible]	Apr. 2[illegible]	Apr. [illegible]	Apr. 1[illegible]	May 13	May 17	Apr. 13	Apr. 2[illegible]	Apr. 2[illegible]	May 6	May 17	Apr. 11	Apr. 2[illegible]	May 6
Nose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pharynx	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0
Right tonsil	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
Left tonsil	0	0	0	0	0	0	a few	0	0	0	0	0	0	0	0	0
Tongue	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

the time of its occurrence. Cultures made almost daily showed this yeast for about ten days, after which it disappeared quite rapidly. Some sort of a biological reaction had clearly taken place—the organism could not have persisted for 10 days without colonizing, but the adaptation was not complete, and consequently elimination was the result.

In the case of the "hemolytic influenza bacilli" we are inclined to assume the above explanation. The organism is not a member of the constant habitual flora, nor was it associated with acute disease or local foci or infection except perhaps in occasional cases. There was no outside source to account for a frequent transient incidence, and yet it was present in nearly 50 per cent of cultures made more or less at random among a mixed group of people.

It seems, therefore, that here we have an instance of an organism which shows incomplete adaptation to growth on the mucous membranes of the upper air passages, although it can exist there in an abortive way without becoming predominant, and independently of the usual accessory factors which explain a carrier state of a foreign organism.

We wish, therefore, to add this possibility to the list of explanations already given of the presence of various bacteria in the throat. Studies from this point of view on the significance of pneumococci and influenza bacilli are now in progress.

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EXPERIMENTAL STUDIES ON HYDROCEPHALUS

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The effect of intravenous injections of solutions of different concentrations on the pressure of the cerebro-spinal fluid was demonstrated for the first time by Weed and McKibben (1919)[1] in their experiments on cats. They showed that such an injection of a concentrated solution of sodium chloride, of other electrolytes and of dextrose, invariably produced a marked reduction of the pressure of the cerebro-spinal fluid, the fall being often below zero. Conversely, they also found that a hypotonic solution (distilled water), given intravenously, was followed by a marked and sustained rise in the pressure of the cerebro-spinal fluid. These results were attributed to osmotic changes taking place in the body-fluids subsequent to the alteration of the salt content of the blood. A change in the actual size of the brain accompanied these alterations in the pressure of the cerebro-spinal fluid. These physiological findings were confirmed in man by Cushing and Foley (1920)[2] and by Ebaugh and Stevenson (1920).[3] Foley and Putnam (1920)[4] not only verified the observations on animals but demonstrated that the same alterations in cerebro-spinal fluid pressure could be brought about by administration of the solutions by mouth or by injection into the intestine. These observations suggested that a series of experiments be undertaken to determine the effect of similar solutions (hypertonic and hypotonic) both on brain bulk and on the pressure of the cerebro-spinal fluid in the dilated cerebral ventricles of hydrocephalic animals. If such changes in the pressure of the cerebro-spinal fluid were observed to follow the intravenous injection of a hypertonic solution it was then planned to investigate, by this means, the interesting problem of absorption of the cerebro-spinal fluid in the closed ventricles of hydrocephalus. For a marked reduction of the pressure of the cerebro-spinal fluid, under these experimental conditions, might well be interpreted to mean an increased absorption of the ventricular fluid.

Method of Experimentation

Artificial Production of Hydrocephalus.—For the success of these experiments the production of an internal hydrocephalus was essential. The typical pathological picture of this type of hydrocephalus has been successfully produced by various investigators in dogs and cats. The experimental procedure employed has concerned usually the occlusion of normal pathways of drainage of the cerebro-spinal fluid, intraventricular or extraventricular; this occlusion has been brought about by mechanical means or by the injection of different substances producing inflammatory reactions which finally blocked the pathways of the fluid. In addition to these methods the low ligation of the vein of Galen has produced different degrees of hydrocephalus.

Dandy and Blackfan (1913,[5] 1914[6]) succeeded in causing internal hydrocephalus in dogs by the occlusion of the aqueduct of Sylvius with pledgets of cotton and also by the ligation of the vein of Galen. Frazier and Peet (1914)[7] reported similar experiments with identical results, after employing similar technical procedures. Thomas (1914)[8] produced internal hydrocephalus in dogs by the intraventricular injection of aleuronate in starch, causing an inflammatory reaction that subsequently occluded the ventricular pathways. Dandy (1919),[17] under the same principle, was able to produce a communicating type of hydrocephalus by the introduction of strips of gauze, saturated with iodine, around the mesencephalon. Other workers such as Burr and MacCarthy (1900),[9] and

Flexner (1907),[10] by the injection of different irritating substances or of a suspension of micro-organisms, produced internal hydrocephalus in kittens and monkeys; in these experiments the primary pathological picture is to be considered an inflammatory meningitis, with the secondary association of the hydrocephalus.

The simple and uninvolved technique of Weed (1920)[11] for the artificial production of internal hydrocephalus was followed in these experiments. The facility with which hydrocephalus of any degree desired was produced within a short time by this method was considered advantageous for the establishment of experimental conditions. This technical procedure for the production of hydrocephalus has been employed with success in these observations, as it also was by Wislocki and Putnam (1921)[12] in studies of the absorption of the cerebro-spinal fluid from the dilated ventricles.

Two methods of equal practical advantage were introduced by Weed for the production of internal hydrocephalus; both employed injections of suspensions of lamp-black. In the first procedure the injection was made directly into the lateral ventricles, while in the second the injection was made into the subarachnoid space at the cisterna cerebello-medullaris through the occipito-atlantoid ligament. Both procedures produced in these experiments typical internal hydrocephalus in kittens, and although both methods were of equal simplicity, we agree with Weed that the subarachnoid route gave a better distension of the ventricles.

A 10 per cent suspension of lamp-black (Germantown Black) in physiological saline solution was used for the injection in all the cases. After the insertion of the sterile needle into the lateral ventricle or cisterna cerebello-medullaris, as much cerebro-spinal fluid as possible was allowed to escape. This was found necessary in order to avoid an excessive intracranial pressure when the lamp-black suspension was introduced; the escape of fluid served also as an indicator that the needle was correctly placed within the ventricle or subarachnoid space. After the release of the fluid 1 c. c. of the suspension of lamp-black was injected very slowly. In some animals, spontaneous respiration was interrupted during the latter stages of the injection; when this occurred, artificial respiration was given without delay. This cessation of respiration was especially frequent in the very young kittens, in which the injection of a cubic centimeter of fluid caused considerable increase in intracranial pressure.

The age of the kittens used for lamp-black injection in this series of experiments varied from 48 hours to 22 days. The age of the animal and the condition of ossification of the cranial vault were important factors in the rate of dilatation of the ventricles and in the degree of enlargement of the head. In very young kittens (one to four days old), diastasis of the cranial vault with distinctly palpable separation of the sutures and enlargement of the fontanelles was noticed as early as the second day after the introduction of lamp-black suspension. In older animals, however, where the union of the bony plates of the skull was advanced and firm, the increase in the intracranial tension did not produce any appreciable enlargement of the head or loosening of the cranial sutures until six or seven days from the time of the lamp-black injection. Marked hydrocephalus, with considerable enlargement of the head, was successfully produced in these kittens, results practically identical with those obtained by Weed. The symptoms of ventricular distension were of a typical character (see Fig. I), and pronounced cases were obtained at the end of the second week after injection.

Recording of Pressure of the Cerebro-Spinal Fluid and Intravenous Injection of Solutions.—In general, the technique described by Weed and McKibben was used throughout. Ether given through a tracheal tube ensured uniform anesthesia both for the control and the hydrocephalic kittens; the method also provided a convenient means for artificial respiration when the necessity arose. The lateral ventricles were entered by an 18-gauge lumbar-puncture needle, with its obturator in place. The puncture was made by passing through the middle of the widely separated fronto-parietal suture. A calibrated U-manometer with a bore of 1 mm., filled with Ringer's solution to the zero point, was quickly attached to the puncture-needle on withdrawal of the stylet. One or more drops of cerebro-spinal fluid were invariably lost in this manipulation; replacement of this fluid by Ringer's solution was made in the manometer. Distinct pulsations of the column of cerebro-spinal fluid due to the heart-beat and respiratory movements were immediately observed in the manometer if the connections were properly adjusted and the point of the needle was free in the dilated ventricle. In these experiments the cardiac pulsations varied from a faint beating of the fluid to 2 mm. excursion, while the respiratory movements were from 2 to 5 mm., *i. e.*, the excursion between the high point reached in expiration and the low point reached in inspiration. The lower point of the respiratory excursion was taken for reading.

Injections of the solutions were all made intravenously. These were given with a syringe by way of one of the femoral veins. In every case administration of the solutions, especially of the hypertonic, was done very slowly with an average rate of 0.7 c. c. per minute.

The body temperature was carefully maintained, during the experiment, by means of an electric lamp and hood. This was of considerable importance in view of the weakened condition of the animals and the length of time required to complete the experiments, occasionally more than two hours.

Physiological Observations

Initial Pressures.—The first level reached by the cerebro-spinal fluid in the manometer, just after its adjustment, was recorded in each case. These initial readings were not considered to be accurate records of the normal pressure, however, because of the unavoidable dislocation of some of the fluid into the manometer and the loss of one or two drops of cerebro-spinal fluid in adjusting the manometer and needle. A second pressure was therefore recorded when the column of fluid attained a fairly constant level, five to ten minutes later; this was taken as the normal initial pressure. Such determinations

were found to average 4 mm. higher than the first. This slight rise of pressure in the beginning probably represents the natural restoration of the fluid displaced and lost in the adjustment of the manometer.

Observations of initial pressures were made on 12 hydrocephalic kittens and on six normal ones. The hydrocephalic kittens were examined from 4 to 20 days after the injection of lamp-black and ranged in age from 9 to 25 days. The average pressure obtained in hydrocephalic cases was 126 mm. of cerebro-spinal fluid. The maximal recorded pressure was 171 mm. and the minimal 100 mm. These pressures, when compared with similar initial readings obtained from normal kittens of approximately the same age and size, were found to be much higher, as might be expected. The maximal recorded pressure obtained in normal kittens was 102 mm., the minimal was 66 mm. and the average was 76 mm. of cerebro-spinal fluid.

Analysis of the initial pressures of the different hydrocephalic animals in regard to age and duration of the artificial lesion failed to reveal any definite relation. There was, however, noticed an important difference between the pressure of the cerebro-spinal fluid in those cases produced by intraventricular injection of lamp-black and those caused by subarachnoid injection. The initial pressure was higher in the former than in the latter, the averages being 141 mm. and 117 mm. respectively. A maximal pressure of 171 mm. and a minimal pressure of 117 mm. were found for the former and 155 mm. and 103 mm., respectively, for the latter. This difference of pressure in the two groups of cases may be explained by the mechanical effect of the lamp-black particles deposited directly against the walls of the ventricles, in those animals subjected to intraventricular injection.

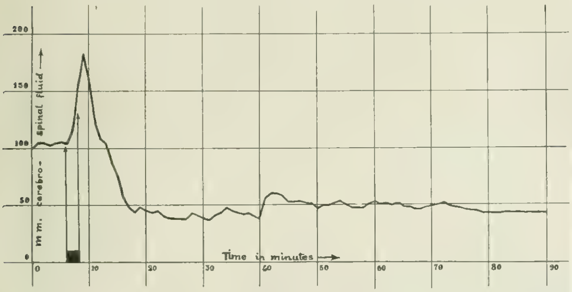

FIG. 1.—Hydrocephalic kitten No. 11. Pressure of intraventricular cerebro-spinal fluid with intravenous injection of 2 c. c. of 30 per cent sodium chloride, during blocked interval.

Hypertonic Solution.—Twelve observations of the effect of intravenous injection of hypertonic solutions upon the pressure of the cerebro-spinal fluid in hydrocephalic kittens have been made in the course of this work and the results have been uniformly constant.

Sodium chloride in 30 per cent concentration was the hypertonic solution used; 2 c. c. of the solution were given in each case. At first the dosage proved frequently fatal, but with proper precautions in the manner and rate of injection and with due regard to the general condition of the individual animal, uniformly good results were obtained.

Examination of pressures of the fluid when carefully plotted showed in general an abrupt initial rise of the pressure of from 10 to 90 mm., usually starting with the beginning of the injection. This rapid rise, in a majority of cases, continued even after the end of the injection, the duration of which was usually about two minutes. The rise was immediately followed by a rapid reduction of the pressure reaching the initial level within three or four minutes and continuing downward until it attained its lowest level. The minimal pressure was usually recorded in from 25 to 30 minutes after the completion of the injection.

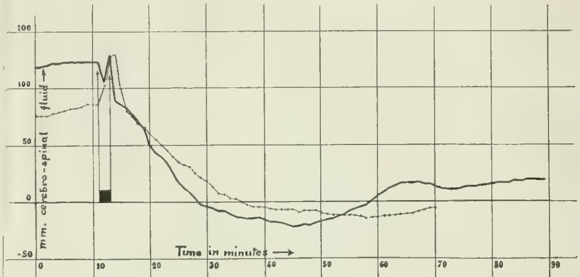

FIG. 2.—Hydrocephalic kitten No. 10 and normal control. Pressure of cerebro-spinal fluid with intravenous injection of 2 c. c. of 30 per cent sodium chloride, during blocked area.

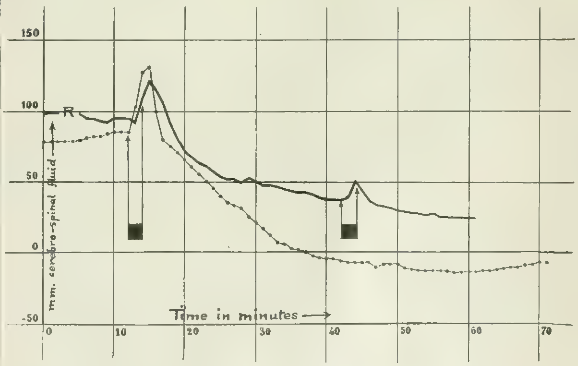

FIG. 3.—Hydrocephalic kitten No. 8 and normal control. Pressure of cerebro-spinal fluid with two separate intravenous injections of 2 c. c. of 30 per cent sodium chloride. The control received but one injection of hypertonic saline. Replacement (R) of the intraventricular fluid with solution of potassium ferrocyanide and iron ammonium citrate was carried out before the hypertonic injection in the hydrocephalic case was given.

Fig. 1 represents a typical curve showing the effect of an intravenous injection of 2 c. c. of 30 per cent NaCl upon the pressure of the intraventricular cerebro-spinal fluid in hydrocephalic kittens. A sudden rise of 87 mm. above the initial pressure was recorded in this case; this increase was soon followed by an equally rapid fall of 150 mm. The minimal

pressure obtained was only plus 37 mm. In some of the cases in this group the cerebro-spinal fluid pressure fell below zero. Fig. 2 shows the chart of one of these animals, a fairly typical curve, which reached a negative pressure of 22 mm. In this case the ascent was not as marked as usually obtained, but the drop was the lowest recorded in the entire series. In Fig. 2 the lowest pressure was reached 30 minutes after the termination of the intravenous injection of salt solution. The return to normal pressure was very gradual, consuming, as in other cases, a period of from two to three hours before reaching the initial level. With this figure is included a control tracing recorded from a normal kitten of the same litter and given an intravenous injection of an equal dose of hypertonic saline solution, the cerebro-spinal fluid pressure being recorded from the subarachnoid space. The time of the injection in the two cases was made to overlap in the plotting to afford a better comparison. In some cases a slight drop of the pressure was noticed just at the start of the intravenous injection, as shown distinctly in Fig. 2; the majority of cases, however, did not show this initial fall.

A characteristic finding in the hydrocephalic kittens subjected to this procedure was the depression of the sutures and fontanelles coincident with the drop of the cerebro-spinal fluid pressure. This was especially marked in advanced cases of hydrocephalus with considerable enlargement of the head, in which the cranial bones were widely separated. As a general rule, the lower the drop of cerebro-spinal fluid pressure, the deeper was the depression along the cranial sutures and fontanelles.

Figs. 3 and 4 are two other tracings showing the typical effect on the intraventricular cerebro-spinal fluid pressure in hydrocephalic kittens of the intravenous injection of hypertonic salt solution. The experimental animal represented by Fig. 3 received two injections of 1 c. c. each of hypertonic solution with an interval of 28 minutes between the two doses. The drop of the pressure, as noticed here, was proportionate to the size of the dose administered. It was also noticed that the effect of the second dose was not so marked as that of the first.

Hypotonic Solution.—Intravenous injection of distilled water, as the hypotonic solution, in hydrocephalic kittens gave a result similar to that obtained by Weed and McKibben in normal adult cats, *i. e.*, a marked rise of the intraventricular cerebro-spinal fluid pressure. This effect on the pressure of the cerebro-spinal fluid was repeatedly obtained in the form of a prompt and enduring rise of the pressure, which usually reached its maximum height from 20 to 30 minutes after the injection was begun. This maximum was in every case maintained for several minutes, ordinarily from 10 to 20, after which the fluid pressure began to fall very gradually, reaching the original pressure two to three hours later.

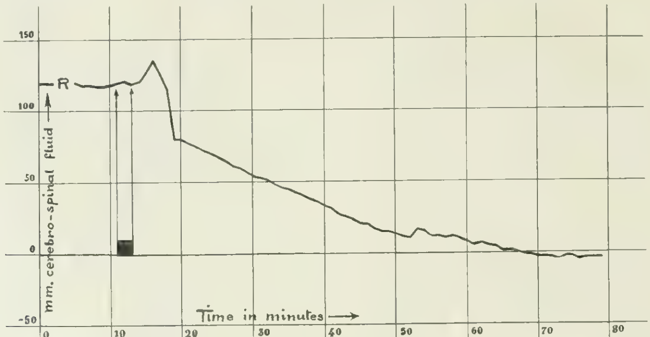

Fig. 4.—Hydrocephalic kitten No. 4. Pressure of cerebro-spinal fluid with intravenous injection of 2 c. c. of hypertonic salt solution after the replacement (R) of the intraventricular fluid with solution of potassium ferrocyanide and iron ammonium citrate.

Fig. 5 is a typical tracing of the changes in the cerebro-spinal fluid pressure following the intravenous injection of

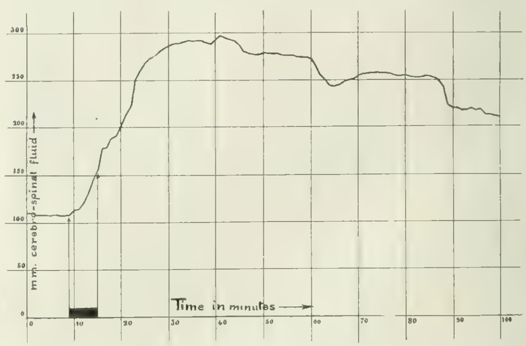

Fig. 5.—Hydrocephalic kitten No. 15. Pressure of the intraventricular cerebro-spinal fluid with intravenous injection of 20 c. c. of distilled water, during blocked interval.

a hypotonic solution. The amount of distilled water used in this case was 20 c. c. injected very slowly into one of the femo-

ral veins. The curve reproduced is only a third of the entire observation made in this particular case. The fall was so gradual that the initial pressure of the fluid was not reached until after a period of three hours. The complete tracing in this case showed a rise in the pressure of the cerebro-spinal fluid to its maximum of 5 mm. per minute in contrast to a fall to normal at the rate of only 0.7 mm. per minute.

Fullness of the head and distension of the open sutures and fontanelles were noticed in these hydrocephalic kittens during the marked rise of the intracranial pressure following the intravenous injection of hypotonic solution.

Absorption from the Dilated Ventricles of Hydrocephalic Animals

At the present time it appears to be fairly well established that the drainage of cerebro-spinal fluid is chiefly by way of the venous system and to a lesser degree also along the lymphatics. From the experimental work of previous investigators it is apparent that attention was mainly directed to the physiological aspects of the problem of absorption and that the detailed anatomical proof of the exact pathway of escape and the definite structures responsible in the absorptive processes have been but partially worked out.

Key and Retzius (1876),[13] whose important studies mark the beginning of modern work on the subject of cerebro-spinal fluid absorption, were the first to demonstrate the passage of colored gelatine, injected into the subarachnoid space, through the Pacchionian granulations into the dural sinuses. They held that cerebro-spinal fluid passed normally from the subarachnoid into the subdural spaces through the granulations and then into the sinuses.

The experiments of Weed (1914)[14] on the absorption of cerebro-spinal fluid have supported the conception of the process set forth in the monograph of Key and Retzius. With the use of solutions of potassium ferrocyanide and iron ammonium citrate and the production of the Prussian blue reaction, Weed concluded that the return of cerebro-spinal fluid from the subarachnoid space to the general circulation is principally by a process of filtration through the arachnoid villi into the great venous sinuses of the meninges. He also agreed that there is an accessory drainage of the cerebro-spinal fluid from the subarachnoid spaces into the lymphatic system.

The results of the observations conducted in man by Dandy and Blackfan (1914)[6] on the absorption of the cerebro-spinal fluid in internal hydrocephalus (obstruction of the channel of exit into the subarachnoid space) suggested clearly that a slow absorption takes place in the cerebral ventricles. They injected phenolsulphonephthalein into the cerebro-spinal fluid and by its quantitative output in the urine they could determine the rate of absorption of the dye from the ventricles. In all the seven cases with completely occluded ventricles that were under their observation the phenolsulphonephthalein was detected in the urine 20 to 45 minutes after the intraventricular injection of this indicator. This result was definite evidence of absorption of the cerebro-spinal fluid from the occluded ventricles of hydrocephalus. They found that the rate of absorption from the ventricles in these cases was from 1 to 2 per cent in two hours; that is, up to 2 per cent of the injected drug was excreted in the urine within the first two hours. In the extensive work of these two investigators they, however, repeatedly asserted that in obstructive hydrocephalus practically no absorption takes place from the ventricles.

The recent experiments of Wislocki and Putnam (1921)[18] on the intraventricular absorption of the cerebro-spinal fluid in hydrocephalic kittens constituted an important step in the solution of the problem of the exact course of escape of the ventricular fluid. For the replacement of the cerebro-spinal fluid in the dilated ventricles, they employed trypan blue and solutions of potassium ferrocyanide and iron ammonium citrate as used by Weed for the Prussian blue reaction. With these two means for identification of the exact course taken in the drainage of the ventricular fluid they were able to demonstrate, anatomically, that absorption occurred to some extent from the ventricles in hydrocephalic animals and that the pathway of escape was through the ependyma into the intercellular spaces and finally into the perivascular spaces. This was a clear anatomical demonstration of intraventricular absorption of the cerebro-spinal fluid in hydrocephalus.

The main object of the following observations was the determination, anatomically, of the structures responsible in the absorption of the intraventricular cerebro-spinal fluid. The physiological proof that such absorption actually occurred was presented in the preceding sections of this report. It is the hope that in this paper are combined the physiological and anatomical studies tending to give clear evidence of this intraventricular absorption of the cerebro-spinal fluid.

Two principal series of observations were undertaken on hydrocephalic kittens to determine the absorptive processes taking place intraventricularly. Similarly, two identical groups of experiments were afterward performed on normal kittens to serve as control and to elucidate further some of the findings in the observations on the hydrocephalic cases. One series of experiments provided for the intraventricular substitution of the cerebro-spinal fluid by a solution of equal parts of 1 per cent each of potassium ferrocyanide and iron ammonium citrate; the substitution of this foreign solution in hydrocephalic and normal kittens was followed quickly by the intravenous injection of hypertonic salt solution. The other series of hydrocephalic and normal kittens was likewise subjected to the intraventricular substitution of the cerebro-spinal fluid by the same foreign solution; then, after letting the animals live for two hours, they were killed without any administration of the hypertonic solution. In both cases all the specimens were soon preserved by aortic injection of fixing fluid consisting of 10 per cent formalin with 1 per cent hydrochloric acid. The addition of the mineral acid caused prompt precipitation of Prussian blue granules *in situ,* giving exact evidence by the distribution of the granules of the course taken by the true solution. Gross and microscopic examinations of the specimens were then made.

The substitution of the cerebro-spinal fluid in hydrocephalic kittens was done by determining the initial pressure of the intraventricular fluid with a manometer connected with the puncture needle. Once the initial pressure was recorded, the manometer was detached from the needle and all the cerebro-spinal fluid was allowed to escape. The volume of the released fluid was determined and then exactly the same amount of the ferrocyanide and citrate solution was introduced. The introduction of the solution was done with a graduated syringe connected with the same puncture needle. The manometer was again inserted and the height of the fluid observed. If the initial pressure was not reached, more of the solution was introduced into the manometer until the original level of the fluid was attained.

The hypertonic solution was given through one of the femoral veins and a dose of 2 c. c. of 30 per cent solution of sodium chloride was usually administered.

In animals in which the hypertonic solution was injected after the replacement of the fluid, a sufficient time was allowed for the reduction of the pressure of the cerebro-spinal fluid, as indicated in the manometer, before the animal was sacrificed. This observation of the physiological reaction permitted one to be certain that absorption had already taken place in the ventricles. For the very same reason, those subjects which were not given injections of the hypertonic solution after the substitution of the intraventricular fluid with the ferrocyanide and citrate solution were allowed to live two hours before they were sacrificed.

In the experiments on normal kittens no observation of initial pressure was made. The substitution of the fluid was performed by the introduction of puncture needles of the same caliber into each of the lateral ventricles. One of these, the more dependent, was used for the escape of the fluid; while the other, the more elevated, was employed for the introduction of potassium ferrocyanide and iron ammonium citrate solution. The introduction of this solution was done by gravity, by means of a small glass funnel connected with a rubber tubing to the higher puncture needle. Very little pressure was used in introducing the solution in this way and free escape of any fluid under abnormal tension in the normal ventricles was assured. This method of replacement did not permit any increase in the normal intraventricular tension. The injection of hypertonic solution in normal kittens was made through the femoral vein and the same dose of salt was administered in all cases.

The use of the Prussian blue reaction with the solution of potassium ferrocyanide and iron ammonium citrate in this experiment was considered to be advantageous; the procedure is the only feasible method that can be used with accuracy in this kind of investigation. It was Weiss (1879)[15] who apparently first introduced this method of precipitating *in situ* such foreign fluids, and since his time many have taken advantage of this reaction in solving the varied problems of anatomy and physiology. Weed has emphasized the manifold advantages of this method over other preparations as a means of studying the processes of absorption of true solutions elsewhere in the body. The precipitation of ferric ferrocyanide (Prussian blue) from the dual solution is effected by the mineral acid combined with the formalin fixative. This precipitate is not affected by the ordinary technique of staining and preparation of histological sections; the characteristic finely granular, blue precipitate can be easily identified under the microscope.

Hydrocephalic Cases with Replacement and Hypertonic Injection.—Macroscopic findings: Gross sections of the encephalon from all of the seven hydrocephalic animals in this group showed the presence of a marked degree of dilatation of the ventricles and thinning of the cerebral cortex. In cases where the hydrocephalus was produced by intraventricular injection of lamp-black the entire ventricles were dark in color throughout except the choroid plexuses which very frequently remained quite free from the lamp-black particles. Animals which received the lamp-black suspension in the cisterna cerebello-medullaris had the ventricles only partially darkened by the black granules.

The distribution of the Prussian blue precipitate was very characteristic in these hydrocephalic kittens and a description of the distribution in one will suffice for all the cases. On section of the specimen, the blue precipitate appeared quite dense in the dilated ventricles, massed against the ependyma wall, and penetrating in zones of diminishing intensity the brain substance. The blue precipitate was most abundant upon and just beneath the lining ependyma of the walls of the lateral ventricles. It gradually became less and less marked towards the periphery or away from the ependyma. The inner grey matter of the brain substance appeared to be the only portion colored with the precipitate; the white cortex was free throughout from the blue coloration. In the region of the basal ganglia and especially within the substance and around the basal attachment of the septum pellucidum the blue precipitate of ferric ferrocyanide was the most abundant. (See Figs. I and II.) The ependyma throughout was also stained blue, though not as intensely as the grey substance just beneath it. An important finding macroscopically was the faint coloration of the choroid plexus with the blue; these structures stood out in contrast to the dark pigment of the surrounding walls.

The wall of the fourth ventricle in some cases was also covered with blue precipitate especially in those cases where the initial injection for the artificial production of hydrocephalus had been made into the cerebello-medullary cistern. The aqueduct of Sylvius was patent in these animals. In three of the seven cases the vein of Galen was found to possess in its lumen some Prussian blue precipitate. In no other situation outside of the ventricles was the ferric ferrocyanide precipitate recognizable.

Microscopic findings: Blocks of brain tissue were taken from each specimen under observation; these were dehydrated in graded alcohols, embedded in celloidin, sectioned and stained for microscopic examination. Stained sections from different regions of the wall of the dilated ventricles were carefully examined to determine the distribution of the ferric

ferrocyanide precipitate and to obtain thereby information regarding the pathway of escape of the ventricular fluid. The ventricular surface of the ependyma was covered here and there with fine blue granules and also with coarser lamp-black particles. (See Fig. III.) Prussian blue in the form of very fine granules was found within the cytoplasm of the ependymal cells and to a certain extent also in between them. The blue precipitate was however most abundant in the tissue beneath the ependymal lining. Under higher magnification these foreign particles were found lying against the external surface of the endothelial wall of the blood capillaries. Very fine precipitates were distinguishable inside the minute capillaries located just beneath the ependymal epithelium. In some of the cases these blood capillaries were so full of this precipitate that the course of the capillary networks could be actually followed by studying the distribution of these granules. (See Figs. III and V.) Farther away from the ependyma the larger vessels did not all show blue precipitate in their lumina. Under closer examination the vessels which possessed blue granules were identified as veins; the arteries were free from the precipitate. It is worthy of note that the veins located in the septum pellucidum and in the ventricular surface of the basal ganglia were in all cases filled with Prussian blue.

These findings give some clue as to the probable course of exit of the ventricular fluid. In connection with the observation of blue granules in the veins it may be remembered that the venae septi pellucidi, usually two in number, empty directly into the small cerebral veins together with the vena terminalis and the vena choroidea. The vena terminalis comes directly from the ventricular surface of the basal ganglia.

In no specimen from this series of animals was the blue precipitate found inside the epithelium of the choroid plexuses; nor were any of the blue granules found inside the capillaries of the choroid plexuses nor in the larger vessels going into or coming out from them. Some precipitate was found over the surface of the choroid epithelium or between the adjoining villi, but never inside the covering epithelial cells. The finding of the precipitate on the outside of the high columnar cells of the plexus was to be expected, but the failure to discover any blue granules within the cell cytoplasm or beneath the cells indicates definitely that no absorption of fluid occurred through these structures.

Hydrocephalic Cases with Replacement Only.—Macroscopic findings: Practically the same gross anatomical features were found in all these cases as encountered in the hydrocephalic animals which had been given hypertonic saline, after the replacement of the ventricular fluid with ferrocyanide and citrate solution. Fig. IV typically represents a horizontal section of the encephalon of one of the cases. It will be noticed that the Prussian blue precipitate is most abundant beneath the ependymal lining. The grey substance of the occipital and frontal lobes was especially filled with dense precipitate. In many cases the septum pellucidum, especially its basal attachment, and the ventricular surface of the basal ganglia were abundantly filled with the blue granules. Such findings coincide fairly well with the distribution of the Prussian blue in the series given hypertonic solution.

Microscopic findings: The cellular protoplasm of the ependymal cells showed the presence of fine blue granules of ferric ferrocyanide. These granules were scattered here and there in the cytoplasm although a greater number were located near the base of the individual epithelial cells. The endothelial walls of the blood capillaries just beneath the ependyma and also to a certain distance away from it were covered with the blue precipitate. Farther from the lining epithelium of the ventricles the small veins could be distinguished from the arteries not only by their morphology but by the presence of the iron precipitate inside their endothelial wall. No Prussian blue granules could be identified within the cytoplasm of the covering cells of the choroid plexuses, nor in the stroma or capillaries beneath these cells. This microscopic picture of the distribution of the blue granules in this series was identical with that found in the first group, with the only minor difference that the amount of Prussian blue found beneath the ependyma, in the grey substance, was less in this series. (See Fig. V.) This indicates that under these experimental conditions the process of absorption was not as active as under the influence of the hypertonic solution introduced into the blood stream.

Normal Cases with Replacement and Hypertonic Injection.—Macroscopic findings: Gross sections of the brains of this control series were made and examined in the same manner as in the previous cases. The normal slit-like ventricles of the brain were clearly outlined by the distribution of the Prussian blue precipitate. The blue tint invaded the brain substance to a distance of 3 to 7 mm. away from the ependyma, gradually becoming fainter towards the cortex. The ependymal lining of the ventricles was colored blue. This normal lining epithelium appeared to be much less prominent than the ependyma of the dilated ventricles in hydrocephalus. The latter was thicker and showed a clear demarcation line from the underlying tissue unlike this normal ependyma, the thickness of which was hardly recognizable with the naked eye. The wall of the fourth ventricle was also stained blue, although not so intensely as that of the lateral and third ventricles. The septum pellucidum and the basal ganglia bordering the ventricles were all colored with the precipitate of ferric ferrocyanide. (See Fig. VI.)

Microscopic finding: The cytoplasm of the lining ependyma showed the blue granular precipitate. Some of the ferrocyanide and citrate solution was precipitated inside the ventricles and the granules were found lying directly against the surface of the ependyma. The blood capillaries beneath it showed the presence in their lumina and against their endothelial walls of the blue precipitate. Farther away from the ependyma in the cerebral grey substance these granules were less in number and were only found in the smallest veins. In the substance of the septum pellucidum and in the portion of the basal ganglia adjacent to the ventricles a considerable amount of the iron precipitate was likewise encountered both

in the cells of the ependyma covering them and in the network of capillaries in their substance.

Normal Cases with Replacement Only.—Macroscopic findings: The same distribution of the Prussian blue precipitate was met with in this normal series as in the hydrocephalic and normal animals already described. The blue tint was localized in the grey substance adjacent to the ventricular ependyma, and especially in the substance of the septum pellucidum and in the basal ganglia. The wall of the fourth ventricle was also colored blue. The apparent difference existing between this and the normal animals which were given injections of hypertonic saline was the far less extensive penetration of the colored solution into the tissues in this present series.

Microscopic findings: The ependymal cells and the underlying capillaries were, as in the previous cases, filled with blue granular precipitate. The number of these granules was, however, considerably less than in the preceding normal series; the penetration of the foreign solution into the cerebral grey substance was also less extensive. The cells of the choroid plexuses were wholly free from the Prussian blue granules.

The gross and microscopic findings in the two groups of normal animals (after replacement of the cerebro-spinal fluid in the ventricles with the solution of potassium ferrocyanide and iron ammonium citrate), one with the intraventricular injection of hypertonic solution and the other without the saline administration, coincided fairly closely with the findings in the two hydrocephalic series. The penetration and distribution of the precipitated ferric ferrocyanide were similar in the two series, but a lesser penetration or absorption of the injected solution was apparent in those animals, hydrocephalic or normal, which were not given an injection of the strongly hypertonic solution.

Discussion

The increased intracranial tension of the cerebro-spinal fluid in hydrocephalus has been known for many years. Many accounts of the intraventricular fluid spurting from the needle on tapping appear in the literature, but many of these statements appear to be exaggerated. Although it is evident that there exists an increased pressure of the cerebro-spinal fluid in hydrocephalus, it is present only in a limited degree. The findings in this study indicate a difference in pressures of the cerebro-spinal fluid in the hydrocephalic and normal control animals, but this difference, while marked, is not extraordinary. The maximal recorded cerebro-spinal fluid pressure obtained in this series of observations was 171 mm.; the average of all was 126 mm. and the lowest was 100 mm. As might be expected the cerebro-spinal fluid pressure of hydrocephalic kittens was higher than the normal pressures obtained from control kittens. The average pressure for the normal kittens in this study was 76 mm., the highest recorded was 102 mm. and the minimal pressure was 66 mm. There existed, therefore, a difference in pressure of 50 mm. of cerebro-spinal fluid between the hydrocephalic and normal kittens.

Compared with the normal pressure of the cerebro-spinal fluid obtained by Weed and McKibben in their experiments on adult cats, the average pressure of the control kittens was lower, while that of the hydrocephalic kittens was much higher. Weed and McKibben gave an average of 119 mm., a maximum of 155 mm. and a minimum of 90 mm. Foley and Putnam[4] gave more variable normal cerebro-spinal fluid pressures for cats with an average of 127 mm., a highest pressure of 255 mm. and a lowest of 65 mm. These determinations of cerebro-spinal fluid pressures of adult cats, when compared with the pressures found in normal kittens, are higher.

The marked dilatation of the cerebral lateral ventricles in the hydrocephalic kittens should be considered as the primary and direct effect of the augmented intraventricular pressure of the cerebro-spinal fluid. This increased pressure within the ventricles is in turn the inevitable result of the damming-back of the fluid into the ventricles, caused by the obliteration of the exits for the intraventricular fluid into the subarachnoid space. These factors and their subsequent results have been discussed by Weed (1920)[11] in his paper on the experimental production of hydrocephalus.

One of the most striking results of the increased dimensions of the lateral ventricles was the extreme thinning of the cerebral cortex. In Fig. I it will be noticed that this process of rarefication took place more in the occipital and parietal regions of the hemispheres than elsewhere, indicating that these are the points of lesser resistance to the internal pressure. The temporal, as seen in Fig. II, and then the frontal regions were the next areas to yield to the increasing pressure of the intraventricular fluid. The basal nuclei appeared to be the least affected in the compression of the ventricular walls. In all specimens the outlines of these nuclei were wholly visible in the floor of the lateral ventricles. Weed described this picture of the dilatation of the ventricles and thinning of the cerebral cortex as closely approximating a partial reversion to the embryonic type of cerebral ventricle.

As the chief pathway of return of cerebro-spinal fluid to the blood stream is outward through the subarachnoid space, it is to be expected that in this artificial production of hydrocephalus there must exist a lack of a normal absorption-rate of the cerebro-spinal fluid. Under these experimental conditions with a normal rate of elaboration of the fluid from the choroid plexuses of the ventricles and with the pathway of escape of the fluid into the subarachnoid space occluded, the normal absorption through the meningeal channels becomes insignificant. Assuming that absorption through the ependymal lining of the ventricles takes place (as demonstrated in these experiments), the normal balance between secretion and absorption of the fluid cannot be maintained, as the subarachnoid pathways of escape are necessary to care for all the fluid produced. The maintenance of a normal equilibrium between the formation and the absorption of the cerebro-spinal fluid requires the patency of the communications between the ventricle and the subarachnoid space.

It should be remembered in this connection that the choroid plexuses have been found in these experiments to be free from

lamp-black granules and from the precipitate of ferric ferrocyanide, both over their lining secretory epithelium and in their substance; it seems more than probable that they are secreting at a normal or slightly reduced rate in these hydrocephalic animals. These observations suggested that the choroid plexus is possessed of only one function,—the elaboration of the cerebro-spinal fluid. There is no evidence that the choroid plexuses take part in the absorption of the fluid from the ventricles.

One of the earliest demonstrations of a slow intraventricular absorption of the cerebro-spinal fluid was presented by Dandy and Blackfan (1914)[6] in the obstructive type of internal hydrocephalus. The method used by them in this experimental work was the introduction of phenolsulphonephthalein into the occluded ventricles and later identification of this substance in the urine. They found that in from 20 to 45 minutes after the introduction of this dye stuff into the closed ventricles, it could be detected in the urine. This finding indicated an absorption of the cerebro-spinal fluid from the occluded ventricles of hydrocephalus. This physiological observation received anatomical support when Wislocki and Putnam (1921)[12] reported somewhat similar experiments in hydrocephalic kittens. In their observations, they made intraventricular injections of trypan blue and the solution of potassium ferrocyanide and iron ammonium citrate. With the microscopic identification of these solutions after fixation, the probable pathway of escape of the intraventricular fluid could be studied; these workers for the first time demonstrated, anatomically, that absorption occurs in hydrocephalic animals through the ependymal lining of the ventricles.

Experiments similar to those of Wislocki and Putnam have been many times repeated in this investigation. The anatomical findings of Wislocki and Putnam showing evidence of intraventricular absorption have not only been definitely substantiated in this work, but a clear physiological proof has also been demonstrated for the first time both in hydrocephalic and normal animals.

Our experiments, in which substitution of the ventricular fluid by an isotonic solution of potassium ferrocyanide and iron ammonium citrate was carried out in hydrocephalic and normal kittens, give additional anatomical evidence of the pathway of exit of the cerebro-spinal fluid from the ventricles. Again, our observations on the alteration of the pressure of cerebro-spinal fluid in hydrocephalic kittens by the intravenous injection of solutions of different concentration offers physiological evidence of an intraventricular drainage of cerebro-spinal fluid.

Gross and microscopic examination of the brains of the experimental animals amply demonstrated that absorption does take place through the ependyma and that the pathway is into the underlying capillaries with drainage into the smaller and then larger venous channels. Such a course of drainage was much more easily made out in those subjects given intravenous injection of hypertonic solution after the replacement of the ventricular fluid.

The manifest drop of the intraventricular pressure of cerebro-spinal fluid in hydrocephalic kittens following the intravenous injection of hypertonic solution of sodium chloride indicates that absorption of the fluid from the ventricle must have taken place. The marked drop of pressure of 100 mm. of cerebro-spinal fluid in many instances in our series of experiments cannot be explained alone by the physical influence of tissue contraction subsequent to the withdrawal of the tissue-fluid. The markedly thinned nervous tissue forming the wall of the dilated ventricles, cerebral cortex and medullary substance, does not seem to have sufficient volume to cause by its shrinkage the marked fall in the pressure of the intraventricular fluid in such hydrocephalic animals. The shrinkage of the brain substance, reported by Weed and McKibben,[16] while marked, hardly seems in these hydrocephalic animals the determining factor in the production of the low and occasionally negative pressures of the cerebro-spinal fluid.

The only plausible explanation for the fall of the cerebro-spinal fluid pressure following intravenous administration of hypertonic saline solution is that of a fairly rapid absorption of the cerebro-spinal fluid from the ventricles through the ependyma into the blood capillary bed. That this absorption does take place by this route is proven by the anatomical findings after the substitution of intraventricular fluid. That this absorption is more rapid after the administration of the hypertonic solution is again supported by the anatomical evidence from the series of experiments in hydrocephalic and normal kittens with replacement of cerebro-spinal fluid by the ferrocyanide and citrate solution, for the colored Prussian blue precipitate has a wider distribution and is more intense in those animals given the injection of hypertonic solution than in the controls. The increased area of the dilated ventricular wall covered by ependyma permits considerable absorption of the fluid under these circumstances; the result of this absorption is apparently shown by the great reduction of the intraventricular pressure, even to the negative values.

Another point of interest brought forth in this series of observations is that absorption of the intraventricular fluid takes place through the ependyma not only in hydrocephalic but also in normal kittens. The experiments conducted in normal kittens with the replacement of cerebro-spinal fluid in the ventricles by the ferrocyanide and citrate solution revealed the fact that slight absorption through the ependyma takes place. It should be mentioned here that a comparative study of the microscopic picture of distribution and abundance of Prussian blue precipitate in the different series of experiments suggested that absorption through the ependyma seems to be rather a slow process unless aided by the intravenous administration of hypertonic salt solution. In the normal animal, also, such intraventricular absorption, while existent, must be of almost minimal physiological importance.

The marked elevation in the pressure of the intraventricular fluid following the administration of hypotonic solution to hydrocephalic kittens was possibly due to the increased volume of the fluid and not to the increased volume of the brain. In the artificially produced hydrocephalus, the choroid plexus

was found free from lamp-black particles and in those cases with replacement of the ventricular fluid by the ferrocyanide and citrate solution no evidence of absorption of the foreign solution by the plexuses was found. Under the influence of the large injection of the hypotonic solution and its accompanying osmotic changes, the apparently normal choroid epithelium may have permitted the passage of more fluid into the ventricle than ordinarily occurs, or fluid may have passed through the ependymal wall into the ventricle. The highly differentiated character of the choroidal epithelium would probably be more efficient in preventing this fluid-passage than would the ependyma, but these hypotheses must remain speculative, inasmuch as no anatomical evidence of the process is available. Another explanation that might be ventured for the rise of the intraventricular pressure is the occurrence of rapid osmotic changes between tissue and blood, leading to the imbibition of fluid by the former with resultant swelling of the brain substance and associated increased intraventricular pressure. The markedly thin cerebral cortex forming the wall of the dilated ventricles cannot in all probability exert much influence in raising the cerebro-spinal fluid pressure to 300 mm. of the fluid as recorded in one of our observations.

From the results in hydrocephalic kittens after the intravenous injection of hypertonic and hypotonic solutions in this series of experiments, it may be concluded that the cerebrospinal fluid in the dilated ventricles may be acted upon by the alterations in salt concentration of the blood, and that the volume of the intraventricular fluid may be apparently increased or diminished by the administration of solutions of different concentrations.

Summary and Conclusions

1. The pressure of cerebro-spinal fluid in kittens in which an internal hydrocephalus has been experimentally produced was considerably higher than that of normal kittens; an average difference of 50 mm. of cerebro-spinal fluid was found in this series of observations.

2. Intravenous injection of a strongly hypertonic solution of sodium chloride in hydrocephalic animals produced a brief initial rise in the pressure of the intraventricular cerebrospinal fluid followed immediately by a marked depression. This decrease in pressure at times produced negative values. This phenomenon is probably to be explained by the apparently rapid absorption of the cerebro-spinal fluid from the dilated cerebral ventricles.

3. Intravenous injection of hypotonic solution (distilled water) in hydrocephalic kittens was invariably followed by a marked and sustained increase in the pressure of the cerebrospinal fluid. This result was possibly due to a rapid elaboration of fluid by the choroid plexuses or to an increased transudation of fluid through the ventricular walls.

4. Intraventricular absorption of cerebro-spinal fluid took place in these hydrocephalic kittens; the pathway of escape was through the ependyma into the underlying capillary network. This absorption of cerebro-spinal fluid was hastened by the intravenous administration of strongly hypertonic solutions.

5. Absorption of the cerebro-spinal fluid was similarly found taking place through the ventricular ependyma in normal kittens. The pathway of escape was the same as described above; the process of absorption was very slow in the normal animal but was hastened by the administration of hypertonic solutions. The physiological significance of this intraventricular absorption in the normal animal is probably minimal.

6. The choroid plexuses took absolutely no part in the intraventricular absorption of the cerebro-spinal fluid.

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DESCRIPTION OF PLATE

Fig. I.—Transverse section of the brain of hydrocephalic kitten No. 8, 22 days after intraventricular injection of 1 c. c. of a 10 per cent suspension of lamp-black. Replacement of the intraventricular cerebro-spinal fluid with solution of potassium ferrocyanide and iron ammonium citrate was followed by intravenous injection of hypertonic salt solution. The black stippling shows the distribution and extent of absorption of the true solution of ferrocyanide and citrate that has been precipitated *in situ*, by the action of mineral acid, as ferric ferrocyanide (Prussian blue). The pressure observation of the cerebro-spinal fluid following the injection of hypertonic salt solution in this kitten is given in text Fig. 3.

Fig. II.—A transverse section of the brain of hydrocephalic kitten No. 4, 13 days after intraventricular injection of 1 c. c. of a 10 per cent suspension of lamp-black. Replacement of intraventricular fluid with the solution of potassium ferrocyanide and iron ammonium citrate was followed by an intravenous injection of hypertonic solution of NaCl. The distribution of the Prussian blue precipitate is shown by the black stippling. The fall of the cerebrospinal fluid pressure after the hypertonic administration is shown in text Fig. 4.

Fig. III.—Drawing under high power of a section through the ependyma and underlying grey substance of the brain from hydrocephalic kitten No. 8. Replacement of intraventricular fluid with ferrocyanide and citrate solution was followed by intravenous injection of hypertonic saline. On the surface of the ependyma are large clumps of lamp-black particles and finer granules of Prussian blue

Fig. I.

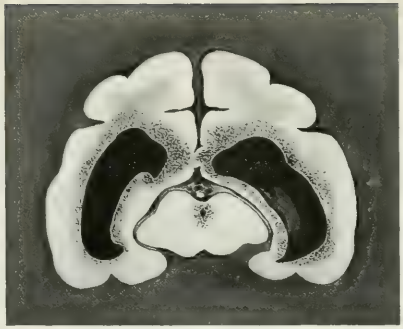

Fig. II.

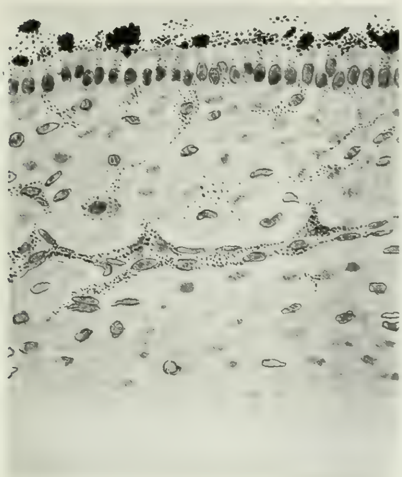

Fig. III.

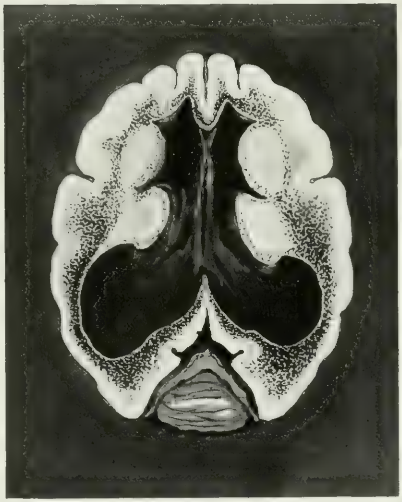

Fig. IV.

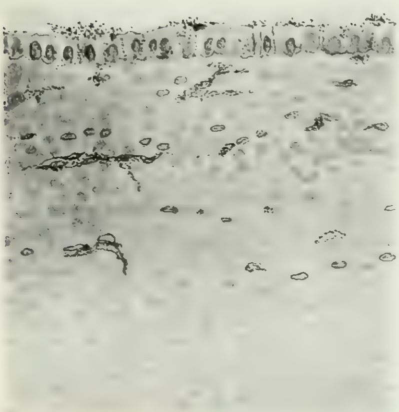

Fig. V.

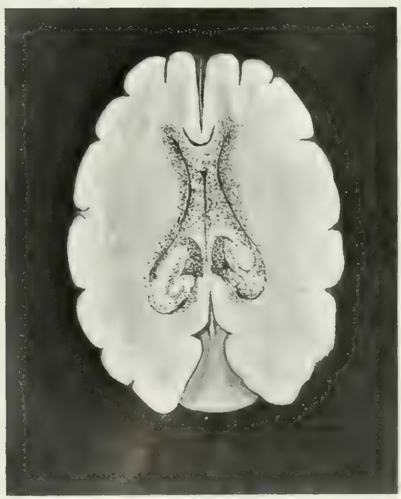

Fig. VI.

precipitate. In the epithelial cytoplasm are seen many fine granules of Prussian blue. The blood capillaries in the grey substance beneath the ependyma are filled with granular precipitate. The larger capillary vessel lower down is full of Prussian blue granules in its lumen.

FIG. IV.—Section of the brain of hydrocephalic kitten No. 11, eight days after the subarachnoid injection of 1 c. c. of a 10 per cent lampblack suspension. Replacement of ventricular fluid with ferrocyanide and citrate solution was carried out and after two hours the animal was killed without any administration of hypertonic solution. The distribution of the precipitate of ferric ferrocyanide in the grey substance is shown by the stippling.

FIG. V.—Drawing under high power of a section through the ventricular wall of the hydrocephalic kitten No. 11. Replacement of intraventricular fluid with potassium ferrocyanide and iron ammonium citrate solution was carried out but without any administration of hypertonic solution of salt. The fine granular precipitate of Prussian blue is found both on the surface and inside the cytoplasm of the ependymal cells. It is present also in the lumina of the blood capillaries of the grey substance under the ependymal epithelium. The penetration of the true solution of ferrocyanide and citrate is less extensive in this case than in the microscopic section shown in Fig. III taken from the hydrocephalic kitten No. 8.

FIG. VI.—Transverse section of the brain of a normal control kitten that had replacement of the intraventricular fluid with solution of potassium ferrocyanide and iron ammonium citrate, followed by intravenous injection of hypertonic solution. The precipitate of ferric ferrocyanide from the foreign solution is found throughout the ventricular walls; it has passed the ependymal lining, has reached the grey substance beneath the ependyma and is within the blood capillaries.

SULPHÆMOGLOBINÆMIA

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The presence of persistent and intense cyanosis which cannot be explained by cardio-vascular or pulmonary disease, by the ingestion of drugs or by the inhalation of gases, is very rare. The first example of this type of case was reported by Stokvis [1] in 1902 under the caption " Enterogenous Cyanosis." The blood of his patient was examined indirectly by applying a spectroscope to the living tissues and a narrow band was seen in the red between the " C " and " D " lines, near the orange, in addition to the usual bands of oxyhæmoglobin. The patient was suffering from severe and prolonged diarrhœa which Stokvis believed to be causally related to the production of the abnormal hæmoglobin derivative.

In the same year Talma [2] reported three other examples of the condition and confirmed the spectroscopic results obtained by Stokvis. In addition he demonstrated by direct examination of the blood serum and of solutions of the blood corpuscles that the hæmoglobin compound was intra-corpuscular and not free in the serum.

Each of these observers believed that the spectra which they had observed were dependent on the presence of methæmoglobin in the circulating blood. Although this assumption was not supported by sufficient evidence, as will be pointed out below, we are, nevertheless, indebted to them for calling attention to this rare type of cyanosis.

Van den Bergh,[3] in 1905, had an opportunity to study two cases of enterogenous cyanosis. The first patient was suffering from a rectal stricture with resulting retention of fæces; the second from a severe chronic diarrhœa. Both showed distinct cyanosis. By careful measurements of the spectra of their blood and by the use of the ammonium sulphide test,* as well as by comparison with the spectra of solutions of artificially prepared methæmoglobin and sulphæmoglobin, he proved that the abnormal pigment in the blood of the first patient was sulphæmoglobin, whereas that in the blood of the second was methæmoglobin.

These results were of fundamental importance, since they demonstrated clearly that two affections easily distinguishable by simple methods may have been included previously under the term " Enterogenous Cyanosis." This designation, therefore, should be abandoned and in its place the terms " Methæmoglobinæmia " and " Sulphæmoglobinæmia " should be substituted according to the nature of the abnormal hæmoglobin compound.

Methæmoglobinæmia occurs not infrequently after the ingestion of certain drugs. It is rare, however, in association with chronic dysentery, as in the cases reported by Stokvis and Talma and in a later case reported by Gibson and Douglas.[4] The associated disease will not be discussed in this paper as we shall limit our report to sulphæmoglobinæmia.

The records of 12 cases of the later disease, in which the diagnosis was supported by sufficient evidence, were found in the medical literature and have been summarized in the accompanying table. A case reported by Jameison [5] and one by Talma [2] possibly belong here, but since definite evidence is lacking, they will not be included. Our present case, therefore, is the thirteenth proved instance of sulphæmoglobinæmia which has been recorded:

A white man 28 years old, a draftsman by occupation, was admitted to The Johns Hopkins Hospital (Med. No. 44858), January 10, 1921, complaining of attacks of weakness during which he turned blue. His father, two sisters and a brother had died of tuberculosis within a few months before his birth. As a child he had had diphtheria, scarlet fever, mumps and measles. At the age of 11 years he was in a hospital for 14 months with hip trouble from which he recovered completely. Since that time he had been well except for rather frequent headaches. He had not suffered from constipation.

His present illness began insidiously in May, 1920, with a sensation of heaviness in the epigastrium, accompanied by weakness and exhaustion. This " all gone feeling " occurred every two to three days and lasted several hours each time. During a more severe attack, in October, 1920, his lips and nails became blue and he suffered from

* This test, apparently not previously known by van den Bergh was described in detail in " The Spectroscope in Medicine," London, 1880, by McMunn.

palpitation and breathlessness. Since that time he has had numerous attacks similar in character but of variable intensity. During the more severe attacks he became very weak and fell but did not lose consciousness. They lasted from two to eight hours and were usually preceded by severe headache and accompanied by trembling, palpitation, marked cyanosis and a peculiar sensation behind the sternum.

Physical examination was made on admission while an attack was receding. There was tremor of the hands and the patient was restless and apprehensive. The nails, lips, tongue, nose and ears and to a less extent the skin in general had a peculiar purple tint as though the patient were under a mercury-vapor lamp. The scleræ were slightly yellow. The tonsils were large, succulent and inflamed. Cardiac dulness extended 10 cm. to the left and 4 cm. to the right of the midline. The sounds were normal and no murmurs were present. There was a slight sinus arrhythmia. The lungs were clear. The fingers were not clubbed. The spleen was just palpable at the costal margin.

Laboratory Examinations.—The erythrocytes numbered 3,584,000 and the leucocytes 13,300 per c.mm. The hæmoglobin was 68% (Sahli). A fresh film showed moderate secondary anæmia and a slight leucocytosis. A differential count was normal. The fragility test showed hemolysis beginning at 0.42% NaCl and complete at 0.32% NaCl. There were 66 vitally stained erythrocytes per thousand. Roentgenograms of the thorax and gastro-intestinal tract were reported normal. The Wassermann test with the blood was negative. Several specimens of fæces showed no abnormalities. The urine showed very strong tests for urobilin at every examination but was otherwise normal. Hemolytic streptococci were obtained in culture from the tonsillar crypts.

Blood drawn during an attack was deep purple-brown in color. On spectroscopic examination of the blood diluted with distilled water, the characteristic band of sulphæmoglobin was present in addition to the bands of oxyhæmoglobin. The spectrum was identical with that of a solution of artificially prepared sulphæmoglobin and slightly different from that of methæmoglobin. Addition of a dilute solution of ammonium sulphide to various dilutions of the patient's blood caused no change in the appearance of the band in the red. That band, however, shifted slightly to the right when illuminating gas was passed through the solution. The patient's serum contained no sulphæmoglobin, and when added to various dilutions of normal red cells or laked red cells it did not convert oxyhæmoglobin into reduced hæmoglobin or sulphæmoglobin, even after long periods of incubation.

The urine and saliva contained no more nitrites than normal and no nitroso-bacilli could be grown from the latter on the medium employed by Wallis.[6]

Treatment.—The tonsils were removed and the patient was given a milk diet and frequent purgation. Bulgara tablets were administered in an attempt to change the intestinal flora. No improvement was noted when the patient was last seen (February 14, 1921).

Clinical Features

A review of the clinical histories of the reported cases is of interest since it shows that the symptomatology has been very similar in each instance. The age of the patients at the time the diagnosis was definitely established varied from 9 to 67 years. However, all but three were past 20 and under 45 years of age. All except two were females. The patients had complained, in most instances, of a variety of symptoms before they recognized the blue color of their nails or lips or suffered from the characteristic attacks. These symptoms included nervousness, weakness, palpitation of the heart, headache and occasionally periods of nausea, vomiting and severe abdominal pain. A few patients had become chronic invalids and after exploratory operation had passed from hospital to hospital for treatment. Constipation was observed in the majority of patients, and in one instance the condition was apparently cured by eradication of a rectal stricture. However, this symptom has been overemphasized since in a number of instances it was not present.

The duration of the vague symptoms enumerated above before the presence of cyanosis was recognized varied from a few months to 12 years. It is difficult, therefore, to judge whether they were dependent on mild degrees of sulphæmoglobinæmia or were symptoms of some other malady. However, they preceded the development of the characteristic attacks so constantly that we believe they may be looked upon as part of the symptom complex.

After the cyanosis had been present for some time the syndrome was usually augmented by the appearance of the more *characteristic attacks* which may be described as follows: After a severe headache of variable duration the patient complains of a peculiar "all gone" sensation behind the sternum. Palpitation, weakness, giddiness and nervousness are present and there may be some dyspnœa. The patient may fall or may actually lose consciousness. His face becomes very cyanotic and he may seem moribund. In some instances the pulse and respiratory rates have been rapid, while in others they have been very slow. The attacks vary in duration and in severity but usually last from two to eight hours. They are followed rather suddenly by amelioration of symptoms and gradually by diminution of the cyanosis.

The intervals between attacks were usually comparatively free from symptoms although some degree of cyanosis persisted and certain vague complaints were present.

Physical abnormalities were seldom observed. The fingers were clubbed in a few instances and occasionally the spleen was palpable. In one case a rectal stricture was present.

Slight secondary anæmia was present rather frequently, usually associated with urobilinuria. The fæces were normal in every instance.

The diagnosis of sulphæmoglobinæmia can be made with certainty by spectroscopic examination as follows:

A small glass container with flat sides about 1 cm. apart is partly filled with distilled water. Whole blood is added to this drop by drop until the two bands of oxyhæmoglobin in the green of the spectrum become very distinct. At this point a definite dark band in the red near the orange (between Frauenhofer lines "C" and "D") will be visible if sulphæmoglobin or methæmoglobin are present in moderate amounts. Otherwise, blood is added until the spectrum, with the exception of the red, orange and yellow, is absorbed. If no band is visible in the red at this point, the diagnosis is not possible by spectroscopic methods.

Unless a large, accurately calibrated spectroscope is used, the ammoium sulphide test must be relied upon to differentiate methæmoglobin from sulphæmoglobin. This test is performed as follows: To the spectroscopic cell containing a dilute solution of blood which shows a band in the red near the orange, a dilute solution of $(NH_4)_2S$ is added drop by drop while the spectrum is observed. If the band in the red persists after a few drops have been added, it is due to sulphæmoglobin. If it disappears, it is due to methæmoglobin.

Another confirmatory test consists in allowing acid-free carbon monoxide to bubble through the diluted solution of blood. If the band in the red and the two bands in the green shift slightly to

the right, sulphæmoglobin is present. If they remain unaltered, methæmoglobin is present.

The blood serum and urine should also be examined for the presence of abnormal hæmoglobin derivatives, since sulphæmoglobin is found only in the red blood cells, in contrast to methæmoglobin which may be found in the serum. Moreover, sulphæmoglobinuria is unknown, whereas methæmoglobinuria following drug poisoning is occasionally observed.

PATHOGENESIS

The mechanism of the production of sulphæmoglobinæmia in man is not known at present, although various observers have established certain facts which have clarified the problem to some extent. Their results will be discussed, therefore, in an attempt to bring the experimental work up to date.

It was recognized by all investigators that the condition was not dependent on the ingestion of drugs or chemicals and that in all probability it was independent of the type of food consumed. Consequently, the cause was sought in some abnormal reaction within the body. Since in many instances constipation was a prominent symptom (and one patient was cured after treatment of a rectal stricture [3]) studies were made to determine whether increased quantities of hydrogen sulphide or an increased amount of hydrogen-sulphide-producing bacteria were present in the bowel. The results of such investigations were not convincing and further attempts to explain the malady by increased production of hydrogen sulphide in the alimentary tract were abandoned.

The suggestion of Steensma [10] that the presence of nitrites in the blood might be the basis of methæmoglobin production was apparently proved by van den Bergh and Gutterink [11] for methæmoglobinæmia. Clarke and Hurtley [7] later demonstrated that the addition of powerful reducing agents, such as nitrites, to blood also greatly accelerated the production of sulphæmoglobin in the presence of minute traces of hydrogen sulphide. Wallis [6] therefore followed these leads in an attempt to elucidate the pathogenesis of sulphæmoglobinæmia. He investigated the saliva of four patients suffering from this condition and in each instance found a nitrite-producing bacillus and increased amounts of nitrites. He demonstrated, furthermore, that the serum of each patient contained a reducing body capable of converting oxyhæmoglobin into reduced hæmoglobin. He assumed, therefore, that the malady was dependent on the absorption by the blood of nitrites from the buccal cavity and of small amounts of hydrogen sulphide from the gut.

This theory is attractive, although the results obtained by Wallis could not be confirmed by Long and Spriggs [12] nor by us, and even Wallis [6] noted that the reducing agent in the blood of his patients seemed more powerful than nitrites.

The problem of the method of the production of sulphæmoglobinæmia in man, therefore, is not solved, although the evidence at hand supports, to some extent, the opinion of van den Bergh that sulphides, formed in the bowel in some manner, gain access to the blood.

The actual amount of sulphide as sulphæmoglobin in the blood of a patient with sulphæmoglobinæmia is not known, but must be very small, since West and Clarke [8] showed that 0.00003 gm. of hydrogen sulphide in 10 c. c. of a mixture of whole blood and water was sufficient to produce a definite spectrum of sulphæmoglobin. This quantity is far below the amount that can be detected by chemical methods, although Meyer [9] had shown that these were 10 times more delicate than spectroscopic tests in demonstrating sulphides in the blood of experimentally poisoned animals. These statements, of course, need confirmation. In sulphæmoglobinæmia in man, however, it is probable not only that minute traces of sulphide are sufficient to produce the abnormal pigment, but also that the sulphides are so firmly secured by hæmoglobin that pas-

Reported by	Reference	Age	Sex	Cyanosis		Spectra	Test with NH_4 2 S
				Duration	Type		
v. d. Bergh	Deutsch. Arch. f. klin. Med., 1905, LXXXIII, 86.	9	M	Several years	Constant but variable.	Band in the Red	Band persisted.
West and Clarke	Lancet, 1907, I, 272	37	F	Two years	Constant	Band in the Red	Band persisted.
v. d. Bergh and Gutterink.	Berl. klin. Woch., 1906 XLIII, 7.	67	F		Marked	Band in the Red	Band persisted.
		60	F		Marked	Band in the Red	Band persisted.
		17	F		Marked	Band in the Red	Band persisted.
Clarke and Curtis	Med. Record, 1910, LXXVIII, 987.	24	F	Few months	Constant but variable.	Band in the Red	Band persisted.
Davis	Lancet, 1912, II, 1145	27	F	Eleven years	Constant but variable.	Band in the Red	Band persisted.
Long and Spriggs	Quart. Jour. Med., 1917-18, XI, 102.	32	F	Two years	Constant but variable.	Band in the Red	Band persisted.
Wallis	Quart. Jour. Med., 1913-14, VII, 73.	27	F	Three years	Constant but variable.	Band in the Red	Band persisted.
		37	F	Four years	Constant but variable	Band in the Red	Band persisted.
Wynter	Proc. Roy. Soc. Med., Clin. Sec., 1908, I, 48-197.	45	F	Twelve years	Constant but variable.	Band in the Red	Band persisted.
Russell and Leathes	Lancet, 1907, I, 659.	30	F	Eight years	Constant but variable.	Band in the Red	Band persisted.
Authors' case		27	M	Two months	Constant but variable.	Band in the Red	Band persisted.

sage of oxygen or carbon dioxide through the blood does not displace them.

The relation of sulphæmoglobin production to the oxygen carrying power of the hæmoglobin has not been determined in man nor in animals, although experiments are being undertaken to elucidate that problem. It seems probable that anoxæmia is partly responsible for the symptoms presented by the patients, although, as is well known, warm-blooded animals die from inhalation of hydrogen sulphide without the production of determinable amounts of sulphæmoglobin.

A few experiments on warm-blooded and cold-blooded animals have been undertaken and may be briefly described here. Lewin[13] administered Schlipp's salt (a compound readily liberating H_2S) to dogs by mouth, subcutaneously, or intravenously and was able to produce the spectrum of sulphæmoglobin. Meyer[9] also succeeded in producing the characteristic spectrum in the blood of a rabbit poisoned by rectal administration of H_2S. The inhalation of hydrogen sulphide by frogs is quickly followed by the formation of sulphæmoglobinæmia. These experiments have thrown little light on the possible mechanism of spontaneous sulphæmoglobinæmia in man.

Treatment

Frequent purging is recommended by all observers and this seems to have relieved the symptoms in some instances. Van den Bergh's patient was cured by operative removal of a rectal stricture. Wallis[6] speaks of cure after vaccine therapy, killed cultures of a nitroso-bacillus being employed. In the majority of instances treatment has failed to afford permanent relief and even the temporary amelioration occasionally observed may have been dependent on rest in the hospital.

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MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE MUSCULATURE OF THE MATURE GRAAFIAN FOLLICLE OF THE SOW

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In the recent literature on the morphology of the ovary, passing mention is made of the occurrence of smooth muscle fibers in the wall of the Graafian follicle. The work reported in this paper was undertaken to verify or disprove these observations, and, if this muscle were proven to exist, to determine the types of autonomic nerve fibers to its cells, and further to investigate the importance of this element in the physiological activity of the follicle.

The authors wish to express to Dr. Corner, of the Department of Anatomy, their appreciation for his generous interest and direction, without which these researches could not have been performed. We also wish to thank Professor Abel and Dr. Macht of the Department of Pharmacology for their advice and help.

History

In the initial volume of his famous journal (1849), *Die Zeitschrift für Wissenschaftliche Zoologie,* von Kölliker was the first to mention smooth muscle as a structural constituent of the ovary. In 1861 C. Aeby by means of the then much relied upon acetic and nitric acid technique made extensive investigations on the character and location of the muscle fibers in the ovaries of various birds and mammals. Two years later Grohe described the complete course of the muscle fibers in the ovary of the pig, from its hilus to the very edge of the

mature follicles, which he found it to encircle.[1] Grohe also mentioned the unusually generous distribution of smooth muscle fibers about the blood vessels of the ovary. In this he supported the hypothesis formulated by Rouget seven years earlier, that, in contracting, the muscle fibers of the ovary compressed the blood vessels, in consequence of which the congestion from impaired venous return led to the rupture of the mature follicles. For a half century little progress was made in either the histology or physiology of the smooth muscle of the ovary; more refined methods of staining were developed but nothing of note was learned. In 1909 H. von Winiwarter and Sainmont announced the occurrence of what they believed to be true smooth muscle fibers in the theca externa of the Graafian follicles of the cat. In the latter part of 1919 Thompson, in his paper on the human Graafian follicle, dwelt at length on the occurrence and purposes of muscle fibers in the theca externa, which he was not able to demonstrate very clearly by a microphotograph. He obtained his best differentiation with safranin and light green. This author stated that the muscular element could not be demonstrated in all of his preparations. Corner, earlier in the same year, briefly mentions the application of the van Gieson technique in distinguishing these fibers. Other than this, the authors have been unable to find mention of the occurrence of muscle in Graafian follicles.

The nerve supply of the Graafian follicle has been a problem long and bitterly disputed. It was early recognized by Frankenhäuser ('67) and Waldeyer ('70) that sympathetic nerves entered the hilus of the ovary with the ovarian artery. This observation was substantiated by all subsequent workers. Riese ('91) showed that the nerve fibers on entering the ovary were separated into two groups—one group accompanying the blood vessels, and the other going directly to the follicles. The workers in this field were soon divided into three camps: Riese ('91), von Herff ('92), von Gawronsky ('94), Winterhalter ('96), Brill ('15) and others believed that nerve fibers independent of the perivascular network penetrate all the layers of the follicle wall, the theca externa, the theca interna and the granulosa. The second group represented by Abel and McIlroy ('12-'13) claimed that only the two outermost coats of the follicle wall have a nerve supply. The third group which numbered among its protagonists Retzius ('93), de Vos ('94), and Mandl ('95) held that the follicle is not in any way penetrated by nerve fibers, other than those of the perivascular network.

Material

Our selection of the sow was based on several considerations. The sow has been found to ovulate spontaneously and to have an œstrous cycle of 21 days. The large size of the ripe follicles (5 to 10 mm. in diameter) offered great advantages, particularly in the physiological work. The existence of two packing houses in close proximity to this laboratory assured us twice daily a supply of fresh tissue. Then too, the experience of Dr. Corner made his advice invaluable as to selection of the ovaries of animals in heat, and the rejection of pathological material.

Our tissue was obtained immediately after slaughter. The ovaries of animals that were in œstrus or just about to enter œstrus formed the chief source of our material. The œstrous period could be determined by the large size of the follicles, the retrogressing corpora lutea, the great vascularity of the peritubal tissue, and the relative pallor of the uteri. Corner ('19), in a study of the follicles of animals known to have been in heat when killed, stated that these follicles possessed clear, translucent, almost spherical walls, protruding a great part of their bulk from the ovary, as is characteristic of the species; they all measured about 7 mm. in diameter, the measurement ranging from 6 to 8 mm. in some which were distorted by crowding. The surface presented no "stigma" or other sign of impending rupture.

The Musculature of the Graafian Follicle

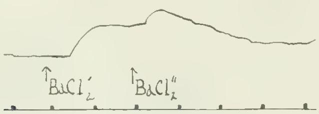
The ovaries used in studying the muscular morphology were promptly immersed in Bouin's fluid. After the Bouin fixation, and the usual paraffin embedding technique, the tissue was sectioned from 3.3 to 5 micra, either by the ordinary microtome technique, or by Professor Huber's wet knife method. The sections were then stained in hæmotoxylin with iron alum as a mordant. After decolorization, they were subjected to the van Gieson technique, for the differentiation of smooth muscle and connective tissue fibers. This stain was found to be entirely satisfactory—the bright red connective tissue and the yellow muscle fibers, with their typical nuclei, forming a brilliant contrast.

In the thecæ externæ of all our specially stained preparations, we were able to distinguish typical smooth muscle cells, with their characteristic cytoplasm and nuclei, which in their distribution gave the suggestion of a lamina over the two internal layers of the follicle, in many places incomplete and interrupted by areas of connective tissue and blood vessels. In many of our sections muscle fibers were found (Figs. 1 and 2), grouped into bundles of rather impressive proportions in juxtaposition to the cells of the theca interna. In many areas these bundles formed pure bands of muscle more than a half-dozen cells in thickness, completely devoid of intercellular connective tissue. In some places, however, only very small scattered groups of muscle cells were present in the follicular wall. Here, as has been noted in the ovarian stroma, these muscle bands seemed to be particularly numerous in the region of the blood vessels. In all of the material sectioned there was no suggestion of the existence of muscle cells in the theca interna. In sections made of a freshly ruptured follicle (the eggs were recovered from the tubes, and two normal, still unruptured, follicles were found in the ovary), the arrangement of the muscle fibers suggests that they played an active rôle in the rupture of the follicle. That the theca externa did not remain as a mere passive membrane on the periphery of the

[1] It is interesting to note that at this time, prior to the great contributions of His ('65) and Waldeyer ('70), the follicular layers had been imperfectly differentiated, the two external layers of the wall of the follicle being generally referred to as the theca folliculi.

newly ruptured follicle, but in conjunction with the other layers projected finger-like processes into the recently contracted follicular cavity, is made evident by Fig. 2. Corner ('19) has shown by means of Weigert's elastic tissue stain, that with the exception of the blood vessel walls the follicle is completely devoid of elastic tissue. Since in this species only about 25 per cent of the recently ruptured follicles contain any blood in their cavities, perhaps, as he suggests, the muscle here by compressing the wall plays the mechanical rôle of a hemostat.

Having followed the advice of Gaskell to found our issue on a firm anatomical basis before resorting to confirmatory physiological measures, we proceeded to prove the existence of muscle by functional tests. We followed the usual "in vitro" technique in this phase of our work. While the ovaries were in Locke's solution needles and thread were passed through opposite poles of the follicle, then by pulling on these sutures we were able to cut the projecting portion of the follicular wall free from the stroma of the ovary. Great care was exercised so that none of the stroma was included in the tissue removed. This bit of living tissue was suspended in a bath of oxygenated Locke's solution maintained at body temperature. The suture



Graph A.—Contraction of wall of Graafian follicle due to stimulation with barium chloride. Time intervals 10 minutes.

$BaCl_2'$ Barium chloride 1-10,000.
$BaCl_2''$ Barium chloride 1-10,000.

on the one end of the follicle was tied to a glass rod well submerged in the solution, while that on the other end was attached to a very sensitive writing lever. In this way accurate records of the muscular contractions of the wall of the follicle could be obtained on a smoked drum. At times in order to make these records more decisive we combined strips of tissue from two follicles of the same ovary.

We first made use of a solution of barium chloride, the classical means of chemically stimulating smooth muscle regardless of its innervation, with which we obtained most active contractions. Even the addition of 2 c. c. of a 0.5 per cent solution of barium chloride to 25 c. c. of Locke answered admirably for this purpose. A relatively long latent period and a gradual protracted "rise" marked the contractions obtained with this drug. With solutions of greater concentrations more marked contractions were obtained than the one recorded in Graph A. At this point we became interested in determining whether the stimulation by barium was due to its toxic effect alone. We found that very definite contractions of the tissue from the wall of the follicle could be obtained by using solutions of barium, made isotonic with Locke's solution, sufficiently dilute to permit the life of amœbæ, paramecia, and rotifers for more than 6 hours. The records which were obtained through barium stimulation were typical of smooth muscle and as barium has not been found to cause the contraction of any tissue other than muscle, we may conclude that the wall of the Graafian follicle contains non-striated muscle-fibers.

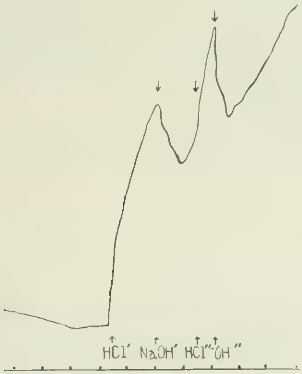
The Nerves of the Graafian Follicle

It is very difficult to stain satisfactorily the nerves of the Graafian follicle. The delicacy of the fibrils makes microtome sections of this material quite unsatisfactory, for at best the sectioned nerve fibers look like a series of fine dots of doubtful structure. It was apparent to us therefore early in our study, that the information we sought could best be obtained from gross material suitably stained. The Golgi and the Bielschowski impregnation methods gave unsuccessful results in our hands, probably because of the thickness of the follicle wall and the impermeability of the outer layers of the ovary. Good results were obtained by Ehrlich's methylene blue staining, following the modifications of J. G. Wilson. Injections of the ovary, through the ovarian or uterine arteries, with a 1/20 per cent methylene blue solution were made under great pressure, which was necessary to insure a complete injection of the mature follicles. After the tissue had been exposed to the oxidation of the air for from one-half to one hour (the time required to stain the nerves distinctly) some of the specimens were fixed in ammonium molybdate, and others in ammonium picrate. The picrate fixation made the specimens easier to tease apart and had the additional advantage of staining the muscle fibers yellow. On the other hand, the tissue after ammonium molybdate fixation cleared much better, and the specimens were made quite firm by dehydration. In specimens fixed in this way it was possible to peel off the various layers of the follicle and study them separately.

In the theca externa, the muscle-containing layer of the follicle, there is a rich plexus of nerve fibers (Fig. 4). The nerves run (10 to 15 together) in good sized non-medullated bundles, independent of the perivascular network which is also demonstrable in our preparations. These bundles of non-medullated nerve fibers give off numerous fibrils to the surrounding tissue. The fibrils have the ordinary morphology of the non-medullated nerve. As Fig. 3B shows, the nerve fibrils course among the muscle cells, running parallel with some cells and crossing others. They give a definite impression of bearing a functional relation to the muscle cells of the theca. These nerve fibrils terminate on muscle cells in sympathetic motor endings (Fig. 3A), the smaller endings covering one muscle cell, while the larger terminations overlie two or three cells. As Fig. 3A shows, these endings are of two distinct types—one ending is large, pale, clear and oval; while the other is small, dark, full of granules and triangular in form.

We have therefore a complete path: nerve fibrils running intimately among muscle fibers, sympathetic motor-endings on muscle cells, and muscle cells capable of functioning. It seems unquestionable that there must be some physiological application for this apparatus. We also thought it desirable to obtain exact information as to the type of autonomic innervation of the ovary and its follicles, since in the abundant

literature on the nerves of this organ we have found no mention of such a study. Thompson ('19) records some pharmacological studies made by Gunn at the former's suggestion, to demonstrate by experimental means the smooth muscle of the ovary. In one experiment conducted on the whole ovary of a virgin rabbit, Gunn elicited a response from stimulation with epinephrine (1-200,000)—an observation which led him to maintain that the ovary has true sympathetic innervation. (It is interesting to note, however, that Gunn finds the true sympathetic nerves to be excitatory nerves of the ovary in the rabbit, while we have found them to be inhibitory nerves in the sow's ovary.) In 1909 under the direction of Von Winiwarter the ovary of the cat was stimulated chemically and electrically but with negative results. Langley and Anderson ('96), in the cat and rabbit, and Kuntz ('19), in the dog, have shown through dissections and physiological degeneration experi-

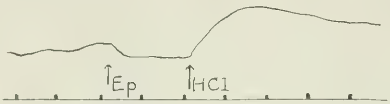


Graph B.—Contraction of the wall of the Graafian follicle due to changes in the hydrogen-ion concentration, caused by the addition of hydrochloric acid and sodium hydroxide.

HCl′ Hydrochloric acid, 0.2 c. c. of 0.3 per cent to 25 c. c. of Locke's solution.

ments that the gonads are innervated by rami from the third, fourth, fifth, and very rarely from the sixth lumbar prevertebral sympathetic ganglia. We have verified this for the sow in a dissection of a large embryo prepared in formalin. However, the important work of Gaskell, Langley and Dickinson, has indisputably shown that the only reliable method of studying autonomic innervation is by means of drugs. By such studies one is enabled to differentiate sympathetic (thoracolumbar) from para-sympathetic (bulbar-sacral) and inhibitory from excitatory fibers. We, therefore, undertook such studies on the nerve supply of the mature Graafian follicle. In this work we followed the ordinary " in vitro " technique already described in this paper. Before the work had progressed far, we noticed that the smooth muscle of the follicle wall is very sensitive to any variation of the hydrogen-ion concentration either to the alkaline or acid side. The addition of such a minute amount as 0.2 c. c. of 0.3 per cent HCl to 25 c. c. of Locke's solution gave the first rise reproduced in Graph B. It was therefore imperative to use neutral salts of the alkaloids or, if no neutral salts were available, to make the solutions neutral with $NaHCO_3$.

On the addition of pure epinephrine, furnished by Dr. Abel, a definite relaxation of the follicle was recorded (Graph C).

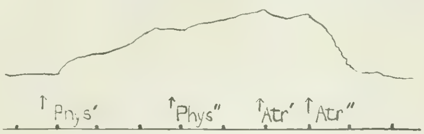


Graph C.—Relaxation of wall of the Graafian follicle due to stimulation with epinephrine and contraction due to the addition of acid.

Ep Epinephrine 1-5000.
HCl Hydrochloric acid.

The extent of the relaxation was practically the same with a 1-10,000 solution as with a 1-2,000,000, the amount of relaxation diminishing slightly with the increased dilution. Thus the nervous mechanism of the Graafian follicle is shown to respond to the action of a drug which stimulates fibers of the true sympathetic system. In this case the action of the drug causes relaxation of the smooth muscle fibers.

Through the addition of physostigmine sulphate (eserine) and atropine sulphate we showed that the follicle also has para-sympathetic innervation. A 1-10,000 solution of physostigmine caused a very marked contraction of the tissue (Graph D). The proof of a para-sympathetic innervation was further substantiated by the action of atropine, which paralyzed the myoneural junction of the para-sympathetic nerve fibers, so that on the addition of physostigmine no contraction was elicited; this drug also relaxed the tissue previously contracted by physostigmine (Graph D). It is clear then that the follicle has a para-sympathetic innervation, these fibers acting as contractors of the follicle wall. Similar results in all respects were obtained by us with experiments on the ovarian stroma—those drugs which stimulate the sympathetic system acting as inhibitors and the para-sympathetic stimulants contracting the ovarian musculature. All



Graph D.—Contraction of wall of Graafian follicle due to stimulation with physostigmine sulphate and subsequent relaxation on the addition of atropine sulphate.

Phys′	Physostigmine sulphate	1-10,000.
Phys″	Physostigmine sulphate	1-10,000.
Atro′	Atropine sulphate	1-1500.
Atro″	Atropine sulphate	1-1500.

of our results demonstrate that the ovary and the follicles of the sow have an innervation analogous to that of the intestines.

All the physiological work reported in this paper was done with the same apparatus, and yet there were marked variations

in the results obtained. With some ovaries the contractions were relatively great; while with others only feeble contractions were noted. From our observations we think perhaps that the amplitude of the contraction depends on the exact moment in the œstrous cycle at which the animals were slaughtered. Those ovaries containing large distended follicles giving the impression of imminent rupture, produced the greatest contractions, while those specimens less advanced in the cycle generally seemed to give contractions of much less magnitude.

Observations Relative to the Method of Rupture of the Follicle

Since the early part of the last century various speculations have been made as to the method of rupture of the Graafian follicle. Suffice it to say that the theories have been almost as numerous as the theorizers. Thompson in 1919 was the first to suggest that the smooth muscle in the wall of the follicle plays a major rôle in this process. It is of interest to note that it had been maintained, as early as 1859, by Pflüger that the peristaltic movements of the frog's ovaries caused rupture of the follicles.

When we found with what readiness the smooth muscle of the Graafian follicle of the sow could be stimulated to contract in vitro, we had high hopes of producing these contractions, with the ovaries still attached to the excised uteri, and thus perhaps induce rupture. We adopted several methods of procedure with the isolated organs—injections of barium chloride, physostigmine, etc., were made into the uterine and ovarian arteries, in some of which high pressure (350 mm. of Hg.) was produced by a hydrostatic apparatus, while in others a low pressure (21 mm. of Hg.) was maintained for a long period of time. Separate follicles were excised from the ovary and direct injections were made into the follicular cavity by means of a hypodermic syringe. Experiments were also performed in which a constant low head of pressure was maintained in these excised follicles, which were immersed in warm oxygenated solutions.

The results of all of these experiments were either negative or so inconstant, because of the experimental difficulties in carrying out these precedures in excised organs some time after death, that we were unable to produce any conclusive evidence as to the rôle of muscle in the rupture of the follicle. We feel confident that experiments conducted on this problem in the living animal would give fruitful results. We believe that our experiments on this phase of the problem have definitely shown that the pure mechanical hypothesis of Hensen which has been so widely accepted, that rupture is induced solely through increased arterial tension, is false. This theory is supported by a single case of rupture induced by Clark ('00) in a human ovary during an intravascular injection of carmine gelatin. In connection with our work 72 injections of the ovarian and uterine arteries of ovaries with mature follicles were made under great pressure. The follicular vessels could be seen to wash out clearly but rupture did not occur in a single instance, even though one braced himself against a wall and pushed the piston of the injection syringe with all of the physical strength available. A constant head of pressure of over 300 mm. of Hg. was exerted through these arteries for hours, but again without inducing rupture. It is indeed difficult to conceive of such enormous pressures existing in the living animal. Dr. Corner tells us that in his previous work in the injections of the follicular vessels of the sow he has never obtained rupture under increased arterial tension.

Conclusions

1. The theca externa of the Graafian follicle of the sow contains an abundance of typical smooth muscle cells.

2. Autonomic nerves with typical motor endings are found in juxtaposition to these muscle cells.

3. The musculature of the ovary and its follicles has a double innervation; the true sympathetics acting as inhibitory and the para-sympathetics as excitatory nerves. This innervation is similar to that of the musculature of the intestine.

4. The rupture of the follicle is not produced solely by an increase in the arterial tension of the follicular vessels.

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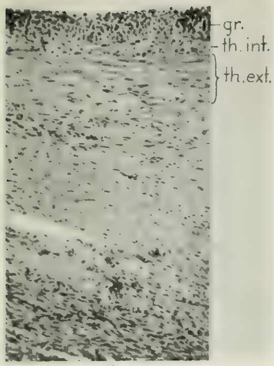

FIG. 1.

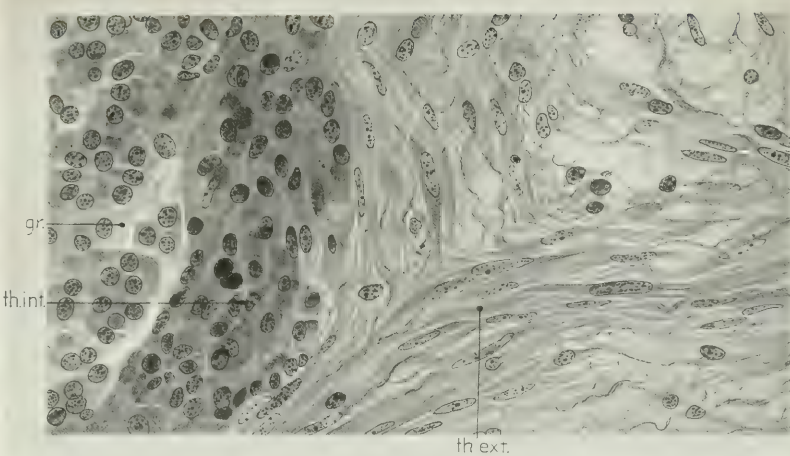

FIG. 2.

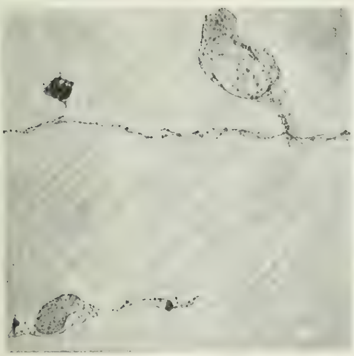

FIG. 3A.

FIG. 3B.

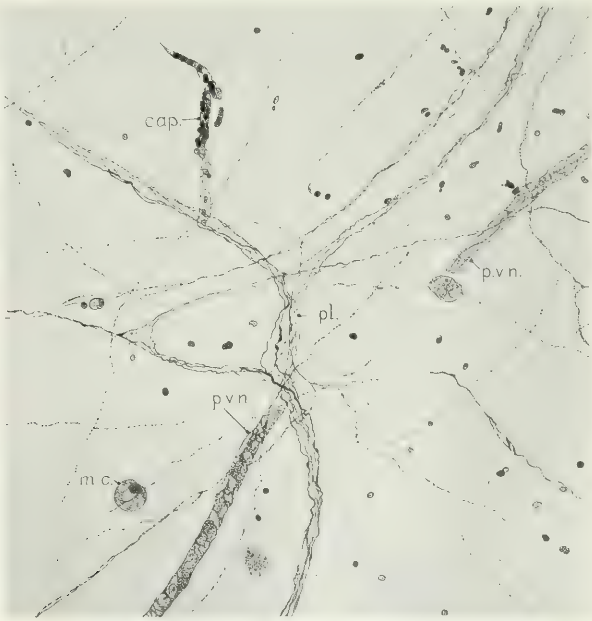

FIG. 4.

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DESCRIPTION OF PLATE

Fig. 1.—Microphotograph of the three normal layers of a mature Graafian follicle of the sow. × 90.

gr. granulosa.
th. int. theca interna.
th. ext. theca externa (the muscle-bearing layer of the follicle).

Fig. 2. Drawing of Graafian follicle immediately after rupture, showing bundles of smooth muscle cells. Van Gieson stain. × 540.

gr. granulosa.
th. int. theca interna.
th. ext. theca externa, containing numerous smooth muscle cells.

Fig. 3a.—Drawing of nerve fibrils with two types of sympathetic motor nerve endings in the theca externa of the follicle. Methylene Blue. × 975.

Fig. 3b.—Drawing of nerve fibril coursing among the muscle cells of the theca externa. Methylene Blue. × 1125.

Fig. 4.—Drawing of teased preparation of theca externa after ammonium molybdate fixation, showing rich nerve plexus and network. Methylene Blue. × 280.

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Tuberculosis of Children. Its Diagnosis and Treatment. By Professor Dr. Hans Much. Translated by Dr. Max Rothschild. 1921. 8°. 156 pages. Macmillan Company, New York.

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Practical Dietetics. With Reference to Diet in Health and Disease. By Alida Pattee. Thirteenth edition. Revised. 1920. 12°. 502 pages. A. F. Pattee, Mount Vernon, New York.

ERRATA

Attention is called to two mistakes on page 342, Plates XLIV and XLV, of the November BULLETIN in the article entitled "The Radiographic Evidence of the Influence of Cod-Liver Oil in Rickets" by Dr. E. A. Park and Dr. John Howland.

Figure 2 should be Figure 1, and Figure 1 should be Figure 2.

Figure 7 should be inverted.

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INDEX TO VOLUME XXXII OF THE JOHNS HOPKINS HOSPITAL BULLETIN

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